**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY**

# DETERMINATION OF PHYSICAL AND MECHANICAL PARAMETERS OF BIOCOMPATIBLE GELATINE FILMS

**M.Sc. THESIS**

**Hacire UĞUR**

**Department of Physics Engineering**

**Physics Engineering Programme**

**OCTOBER 2015**

**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY**

# DETERMINATION OF PHYSICAL AND MECHANICAL PARAMETERS OF BIOCOMPATIBLE GELATINE FILMS

**M.Sc. THESIS**

**Hacire UĞUR**
**(509081118)**

**Department of Physics Engineering**

**Physics Engineering Programme**

**Thesis Advisor: Assoc. Prof. F.Gülay ACAR**

**OCTOBER 2015**

**İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ**

# BİYOUYUMLU JELATİNLİ FİLMLERİN FİZİKSEL VE MEKANİK ÖZELLİKLERİNİN BELİRLENMESİ

**YÜKSEK LİSANS TEZİ**

**Hacire UĞUR**
**(509081118)**

**Fizik Mühendisliği Anabilim Dalı**

**Fizik Mühendisliği Programı**

**Tez Danışmanı: Doç. Dr. F.Gülay ACAR**

**EKİM 2015**

**Hacire Uğur**, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology** student ID 509081118 successfully defended the **thesis** entitled "**DETERMINATION OF PHYSICAL AND MECHANICAL PARAMETERS OF BIOCOMPATIBLE GELATINE FILMS**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

**Thesis Advisor :** **Assoc. Prof. F.Gülay ACAR**
İstanbul Technical University

**Jury Members :** **Assoc. Prof. F. Gülay ACAR**
Istanbul Technical University

**Assoc. Prof. Sevim İşçi TURUTOĞLU**
Istanbul Technical University

**Assoc. Prof. İpek Kanat ÖZTÜRK**
Istanbul University

**Date of Submission : 30 September 2015**
**Date of Defense : 27 October 2015**

***To my father,***

## FOREWORD

This master thesis is written as completion to the my master in physics at the Istanbul Technical University. First of all I would like to thank my advisor Assoc. Prof. F. Gulay ACAR for guiding and supporting me everytime and all conditions. She has set an example of excellence as a researcher, mentor, instructor and role model. This work was founded by BAP project of Istanbul Technical University with the project number of BAP-38678.

I am grateful to Assoc. Prof. Gülşen A. EVİNGÜR to had let me to use her laboratory for the tensile test experiment. I would like to thank also Prof. H.Huceste GIZ for all her guidance through this process, her discussion, ideas and feedback has been absolutely invaluable.

I would also like to thank my amazing family for the love, support and constant encouragement I have gotten over the years. In particular, I would like to thank my lovely father Remzi UGUR. He is the salt of earth and I undoubtedly could not have done this without him. Finally I would like to thank my dear friend Begum Inal TURNA to encourage me every part of my study.

October 2015 Hacire UĞUR

(Physicist)

**TABLE OF CONTENTS**

**Page**

## ABBREVIATIONS

| | |
|---|---|
| **ASTM** | **:** American Society for Testing and Material |
| **SI** | **:** International System of Units |
| **P** | **:** Poise |
| **cP** | **:** Centipoise |
| **Pa.s** | **:** Pascal-second |
| **N** | **:** Newton |
| **m** | **:** meter |
| **kg** | **:** kilogram |
| **mPa-s** | **:** milli Pascal-second |
| **cSt** | **:** Centistokes |
| **St** | **:** Stokes |
| **Pa** | **:**Pascal |
| **c.g.s** | **:**centimeter gram second |
| **UV** | **:**Ultraviole |
| **rpm** | **:**rotation per minute |
| **RF** | **:**Riboflavin |

## LIST OF TABLES

**Page**

**LIST OF FIGURES**

**Page**

## DETERMINATION OF PHYSICAL AND MECHANICAL PARAMETERS OF BIOCOMPATIBLE GELATINE FILMS

## SUMMARY

Biocompatibility may be defined as the ability to respond of the material applied to the body respect to the body system. Biocompatible material which is natural or synthetic materials that can be used for the purpose of fulfill the function of tissues in vivo organisms or to support the deteriorating tissue. The hydrogels that are used as corneal bandage or artificial leather for to cure the deformation of the bones hydroxyapatite, are biocompatible materials. The use of biocompatible materials in medical treatments gives positive results in medical therapies at recent years.

Biocompatible films are crosslinked polymeric structure and they have wide range of medical applications. Owing to the effective and successful results in medical applications, studies about the biocompatible films have remarkable significance during the late years.

Biocompatible film research area has several advantages regarding to medical aspect such as no toxin release in vivo metabolism, not having carcinogenic effect, being chemically stable material, existing in vivo organism as long as it maintains its lifecycle, having appropriate density and weight properties; economical aspect such as being accessible; manufacture aspect such as convenience process.

The main aim of this thesis is to determine appropriate mechanical strength of biocompatible solutions and films and physical parameters respect to medical applications. Gelatin was chosen as a based biocompatible material. Various concentrations of gelatin were determined such as 5%, 3.3%, 2% ,1.42%, 1% and 0.6%. Films were prepared using these solutions. Viscosity measurement were done for solutions and tensile test were done for films and results of each experiments were analysed to obtain mechanical results for each material. After analysing results of experiments two concentrations of pure gelatin solution 5% which was most concentrated and 2% which had convenient results were chosen to observe influence of riboflavin, glucose and NaCl. Concentration of glucose and NaCl were altered between 1% to 5% at a rate of 1% increment. Films were prepared using these solutions. Viscosity measurement and tensile test were performed for each concentration too. Besides the glucose and NaCl riboflavin was added to pure gelatin concentration to observe affect of riboflavin which is UV sensitive material.

The viscosity values and flow behaviour of each solution and Elasticity Modulus of each film were determined after experimental procedure. Viscosity and flow behavior of solutions were related with mechanical properties of fluid form and Elasticit Modulus was related with mechanical properies of solid form of each sample. These parameters were used in order to determine which material is appropriate for biomedical application.

One of the significant implementation of this thesis is preparation of biocompatible UV sensitive films which can be used to gathering strength properties, by making crosslinking bounding, of the tissues such as collagen structural cornea, skin or arteries that loses their elasticity and usage of this thin films in the availability of bioprosthesis. The film samples were prepared by using with and without biocompatible UV sensitive materials. The physical parameters of the mentioned samples were specified by tensile tests, viscosity measurements and pH measurements.

# BİYOUYUMLU JELATİNLİ FİLMLERİN FİZİKSEL VE MEKANİK ÖZELLİKLERİNİN BELİRLENMESİ

## ÖZET

Biyouyumluluk canlı bir sisteme implante edilen, organ ve dokuların işlevlerini kısmen veya tümden üstlenmek üzere tasarlanmış malzemelerin canlı sistemine fiziksel, kimyasal, biyolojik uyumu ve sistemin mekanik davranışına sağladığı uygun cevap verebilme yeteneği olarak tanımlanabilir. Bu amaca uygun olarak tasarlanan ve sistemdeki canlı dokuların işlevlerini yerine getirmek veya desteklemek amacıyla kullanılan doğal yada yapay malzemeler de biyoyumlu malzemeler olarak tanımlanabilir. Biyomalzemelerin mekanik özellikleri, sistem ile uyuşabilirliği yani biyouyumluluğu, korozyona dayanımı, üstün sürtünme ve aşınma dayanımı göstermesi önemli parametrelerdir. Biyomalzemelerin, üstün mekanik özelliklere ve biyouyumluluğa sahip olmaları gerektiğinden seçilen malzemenin kullanım yerine göre uygun özellikleri taşıması oldukca önemlidir. Vücudumuzdaki kemiklerde oluşan şekil bozukluklarının tedavisinde kullanılan hidroksiapatit, kornea bandajı veya suni deri olarak kullanılan hidrojeller biyouyumlu malzemelere ve kullanım alanlarına örnek olarak verilebilir. Tıbbi tedavilerde biyouyumlu malzemelerin kullanılması son yıllarda hastalıkların tedavisi için oldukça olumlu sonuçlar meydana getirmiştir.

Biyouyumlu filmler geniş tıbbi uygulama alanı olan biyouyumlu malzemelerdir. Genel olarak çapraz bağlı polimerik yapılardır ve tıbbi uygulamalardaki etkin ve başarılı sonuçları nedeniyle biyouyumlu filmler ile ilgili çalışmalar son yıllarda oldukça önem kazanmıştır. Tıbbi açıdan; canlı metabolizması içinde yer aldığında toksin salgılamaması, kansorejen bir etkiye yol açmaması, kimyevi anlamda stabil malzamelerden olması, canlı yaşamını sürdüğü müddetçe canlıyla beraber var olabilmesi, yoğunluğunun ve ağırlığının metabolizma için uygun olması; ekonomik anlamda ulaşılabilir olması; üretim açısından işlenme kolaylığı göstermesi biyouyumlu filmleri araştırma alanı olarak oldukça cazip kılmıştır.

Yapılan bu tez çalışmasında, biyouyumlu filmlerin uygun mekanik mukavemetinin belirlenmesi ve kullanılan tıbbi uygulamaya göre fiziksel parametrelerinin belirlenebilmesi amaçlanmıştır.

Bu tez çalışmasınıin deneysel aşamasında öncelikle biyouyumlu malzemeler kullanılarak solüsyonlar hazırlandı ve bu solüsyonlar kullanılarak biyouyumlu filmler yapıldı. Biyouyumlu malzemelerin fiziksel özellikleri; solüsyon halinde iken viskozluk ölçümü ile film halinde ise çekme deneyi ile iki aşamada belirlendi.

Viskozite, solüsyonun herhangi bir yüzey gerilimi altında deforme olmaya karşı gösterdiği direnç olarak tanımlanır ve solüsyonun herhangi bir akış esnasında tabakaları farklı hızlarda hareket eder. Solüsyonun viskozitesi, uygulanan kuvvete karşı direnç gösteren tabakalar arasındaki yüzey gerilimlerinden dolayı ortaya çıkar. Solüsyonun tabakaları arasındaki yüzey gerilimi ile tabakalara dik yöndeki hız

gradyeni liner bir orantıya sahip ise solüsyonun viskozitesi Newtonian, değil ise non-Newtonian olarak adlandırılır. Solüsyonların viskozite ölçümleri rotasyonel viskozimetre kullanılarak yapıldı. Ölçüm sonuçları kayma gerilimine karşılık kayma hızı grafikleri ile gösterilerek solüsyonların akma davranışları incelendi.

Biyouyumlu solüsyonlar kullanılarak hazırlanan filmlerin fiziksel parametreleri çekme deneyi yapılarak belirlendi. Uygun ölçülerde dikdörtgen şeritler halinde kesilen filmlere deformasyona uğrayana dek tek eksende çekme kuvveti uygulandı. Çekme deneyinden elde edilen gerilme-deformasyon değerleri grafiksel olarak gösterildi. Filmlerin elastik şekil değiştermesinin ölçüsü olan Elastisite Modulü gerilme-deformasyon grafikleri kullanılarak belirlendi.

Biyouyumlu özelliklere, deneysel çalışmalarda kullanım kolaylığına ve düşük maliyete sahip olduğundan dolayı biyouyumlu malzeme olarak jelatin, filmlerin temel malzemesi olarak seçildi. Öncelikle uygun konsantrasyonun belirlenebilmesi için; %5, %3.3, %2, %1.42, %1 ve %0.6 konsatrasyonlarında jelatin solüsyonları aynı ortamda ve çevresel koşullarda hazırlandı. Sıcaklık mekanik özellikleri etkileyen önemli bir parametre olduğundan laboratuvar sıcaklığı $24^0$C sabit tutuldu. Hazırlanan solüsyonların biyouyumlulu olabilmesi için doğal polimerik bir malzeme olan jelatinin seçilmesi dışında pH ölçümleri her solüsyon için ayrı ayrı yapıldı. Bu solüsyonlar kullanılarak filmler hazırlandı. Solüsyonlar için viskozluk ölçümü, filmler için çekme deneyi yapıldı ve her bir malzemenin mekanik özelliklerinin belirlenmesi için deney sonuçları analiz edildi. Deney sonuçlarının analiz edilmesinden sonra en yoğun konsantrasyona sahip olan %5 ve uygun parametrelere sahip olan yani viskozite ölçümü sonucu Newtonian davranış gösteren %2 konsantrasyonlu solüsyonlar filmlerin ana malzemesi olarak belirlendi.

Çalışma farklı biyouyumlu malzamelerin jelatin solüsyonlarının ve filmlerinin mekanik özellikleri üzerindeki etkilerini inceleyebilmek amacıyla genişletildi. Riboflavin, glikoz ve NaCl gibi doğal biyouyumlu polimerik malzemeler seçildi ve bu malzemelerin temel yani baz malzeme olarak belirlenen %5 ve %2'lik jelatin solüsyonlarının ve bu solüsyonlardan hazırlanan filmlerin mekanik özelliklerine etkileri incelendi. İlk olarak farklı konsantrasyonlarda NaCl ve glikoz çözeltisi hazırlandı. Glikoz ve NaCl konsantrasyonları %1 ile %5 arasında %1 artış oranıyla 5 farklı çözelti şeklinde hazırlandı. Hazırlanan her bir NaCl ve glikoz çözeltisi %20 oranında, %5 ve %2'lik jelatin solüsyonları ayrı ayrı %80 oranında alınarak jelatin bazlı tuz ve glikoz solüsyonları hazırlandı. Bu solüsyonlar kullanılarak filmler hazırlandı. Her bir konsantrasyondaki malzeme için viskozluk ve çekme deneyleri yapıldı. Ölçüm sonuçlarından elde edilen veriler kullanılarak NaCl ve glikozun, jelatinin solüsyon halinde ve film halinde mekanik özelliklerine etkileri, solüsyon halinde iken viskozluk değeri ve akma davranışı ile film halinde iken Elastisite Modulü değeri ile gösterildi.

Glikoz ve NaCl dışında, riboflavin saf jelatine eklenerek UV duyarlı bir malzeme olan riboflavinin etkisi incelendi. %0.05 konsantrasyonunda riboflavin çözeltisi hazırlanarak bu çözelti %20 oranında ve jelatin solüsyonu %80 oranında alınarak jelatin bazlı riboflavin solüsyonları ve filmleri hazırlandı. Riboflavin UV duyarlı biyouyumlu polimerik bir malzeme olduğundan hazırlanma sürecinde ışık temasının olmamasına özellikle dikkat edildi ve oda sıcaklığı $24^0$C olarak sabitlendi. Jelatin bazlı riboflavin solüsyonu için viskozluk ölçümleri ve jelatin bazlı riboflavin film için çekme deneyi UV ışık öncesi ve sonrası olmak üzere iki aşamada yapıldı. Ayrıca UV süresinin etkilerini inceleyebilmek için 15 dakika, 30 dakika, 45 dakika ve 60

dakikalık 4 farklı sürede UV ışık gören jelatin bazlı riboflavin solüsyon ve filmlerinin viskozluk ve çekme deneyleri yapıldı.

Yapılan bütün deneysel çalışmalardan elde edilen veriler grafikler halinde hazırlandı. Mekanik parametreler, viskosite ve Elastisite Modulü grafikler aracılığıyla belirlendi.

Kollajen yapıya sahip olan kornea, deri ya da damar gibi dokularda çapraz bağlanma yaparak elastikliğini kaybetmiş olan bu dokuların elastikliğini tekrar kazanabilmesi için biyouyumlu UV duyarlılığı olan film hazırlanması ve bu filmlerin biyoprotez kaplamada kullanılabilirliği yapılacak olan bu yüksek lisans tezinin önemli uygulamalarından biridir. Hazırlanan solüsyon ve film numuneler, biyouyumlu UV duyarlı malzemeler kullanılarak ve kullanılmadan oluşturuldu. Hazırlanan biyouyumlu numunelerin fiziksel parametreleri çekme deneyleri, viskozite ölçümleri, pH ölçümleri ile belirlendi.

## 1. INTRODUCTION

The purpose of this study is researching and determining the mechanical properties of pure gelatin solutions and films besides the effect of biocompatible material on mechanical strength of gelatin solutions and films. Gelatin was chosen for based material because it has remarkable biocompatible characteristics and it is easy to use in experimental study besides the cost of gelatin is low to compare other chemicals. Another reason why gelatin was selected is that gelatin has broad applications as cell-sheet carriers and it is used as bio-artificial equipments in medical treatments [1]. For these reasons first of all the impact of concentration on physical characteristic of gelatin solution was studied. Various types of concentrations were selected such as 5%, 3.3%, 2%, 1.42%, 1% and 0.6% ($\frac{\text{weight}}{\text{volume}}$). The viscosity measurement of solution and the tensile test of films were done for each pure gelatin concentration and then viscosity of each solution and elastic modulus of each film were determined. In this way the physical parameters of each pure gelatin concentration in solution form and film form were defined. 5% and 2% concentrations of gelatin were selected as base material. Because of the fact that the 5% gelatin concentration is most intensive one and 2% gelatin concentration has suitable mechanical characteristic as solution form and film form.

The mechanical properties of gelatin in combination with natural biopolymers like riboflavin (RF), salt (NaCl) and glucose were measured. 80% percentage of base gelatin and 20% percentages of natural biopolymers were mixed. The amounts of NaCl and glucose were varied 1% to 5% as increment of 1%. Gelatin combinations of natural polymers were prepared in solution form and film form. The viscosity measurement of solution form and tensile test of film form were done for each concentration. After that physical parameters of combinations were defined as viscosity and elasticity modulus.

All preparations and measurement were done at $24^0$ C and pH values of each solution were measured which is important parameter in biocompatibility. To define physical

parameters of each pure gelatin materials and gelatin-based materials have critical impact on how these materials have been used in medical treatments. Besides gelatin-based materials have been evaluated for tissue engineering and regenerative medicine, innovations will prompt research of several modifications of gelatin-based materials [1].

In this work theoretical background was explained in Chapter 2. Explanation of biocompatibility and biocompatible materials were clarified in this chapter. After that in Chapter 3 applications of biomaterials were demonstrated by three examples which have significant impact on scientific work. In Chapter 4, theoretical explanation of mechanical parameters such as viscosity, elasticity and elastic potential energy which were determined for all materials during this thesis were defined. Materials and their chemical and physical properties were explained in Chapter 5. In Chapter 6 experimental procedure were clarified sequentially. In the last chapter results of this thesis and recommendation were demonstrated.

## 2. BIOCOMPATIBILITY

### 2.1 Definition of Biocompatibility

Biocompatibility is related with capability of material behavior in suitable host reaction in implanted part of human organism. The definition of biocompatibility was discussed in various scientific studies and it was firstly defined in Dorland`s Medical Dictionary [2]. According to definition in Dorland`s Medical Dictionary the term of biocompatibility means the characteristic of materials which does not impact on biological organism as toxically and does not damage on it [3].

The main concern about biocompatibility is that material which was implanted in body part has not undesirable effects. Several parameters are defined in being biocompatible for materials. First of all implantable devices stay in living creature during the live so that least toxic material is chosen as implantable devices in medical treatments besides the performance of material. Secondly biocompatibility is defined as material properties but also it is related with these materials are used in which part of body since materials performance would be varied for each part of body. Another important parameter for biocompatibility is that inert materials are accepted by living organisms or ignored. Last but not least important parameter about biocompatibility is that implanted material could be remaining in living organism during its live [4].

### 2.2 Biocompatible Materials

During the recent years important advances have been in to produce artificial implanted devices which have replaced injured body parts. The main aim of many scientific works is to improve and characterize implanted devices which are used in human body and improve the quality of life. Basic parts of implanted devices are biomaterials which are used to produce these devices. Biomaterial or biocompatible material is synthetic or natural material which is used in medical treatment and

replaces any part of biological organism. To produce biocompatible devices and using biocompatible materials have a wide aspect in science such that physics, material science, chemistry, medicine and so on [Url-1]. For instance, biocompatible, flexible and hydro gel material is used to produce artificial cornea in March 2004 by Dr. Ming Wang who writes his name in history of medicine [5].

Biocompatible materials generally divide into three categories;

- Metals such as stainless steel which is used as implants to provide stability and restore initial bone mechanics also speed up the healing process.
- Ceramics such as aluminum oxide is used as a dental restorative material.
- Polymers such as silicones are used as pacemakers or hydrocephalic shunts. Natural polymers such as gelatin are used in ocular tissue engineering.

The main concern of this thesis is natural polymers so that only natural polymers were explained detail.

### 2.2.1 Polymers

A polymer is a substance which is formed by many repeated subunits. Polymers vary on what kinds of molecules are formed and how they are combined. The basic part of polymer is called monomers and they are joined sequentially, forming in chain. In some types of polymers only one monomer is combined and other type two or three different monomers are combined [Url-2]. The word of polymer is combination of Greek words poly which means many and mer which means part [Url-3]. The process of combining monomers together to produce a polymer is called polymerization. The measure of molecular weight which is given by the number of repeat units in the chain is characterized by degree of polymerization. The distribution of lengths and degree of polymerization determine many important properties of polymer molecule. The number average ($M_n$) and weight average ($M_w$) molecular weight are two parameters which characterize the distribution of polymer lengths of material [Url-4]. $M_n$; the number average molecular weight is evaluated from the mole fraction distribution of different sized molecules in a material. $M_w$; the weight average molecular weight is evaluated from the weight fraction distribution of different sized molecules in a material [6].

$$M_n = \sum n_i M_i \tag{2.1}$$

$$M_w = \sum w_i M_i \tag{2.2}$$

$n_i$ = mole fraction of chains with molecular weight $M_i$

$w_i$ = weight fraction of chains with molecular weight $M_i$

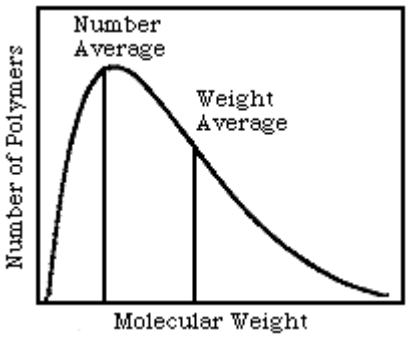



**Figure 2.1:** Typical distributions of polymers including the weight and number average molecular weights [Url-4].

The degree of polymerization also has a great impact on the mechanical properties of a polymer. For instance, the viscosity of polymer in solution is defined by the molecular weight of a polymer. Besides the viscosity, ductility, tensile strength and hardness of solution increases when the chain length of polymer increases. This is illustrated in Figure 2.

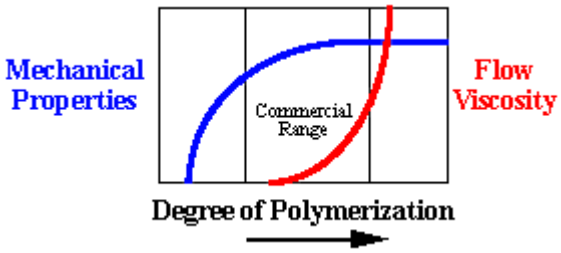



**Figure 2.2:** The degree of polymerization effect on the mechanical properties of a polymer [Url-4].

Polymers generally are categorized into synthetic and natural polymers according to formation of monomer types. Natural polymers such as gelatin, glucose, salt (NaCl) and RF were used experimental part of this thesis [Url-2].

#### 2.2.1.1 Biopolymers

Biopolymers are polymers which are produced by natural organisms such as plants and animals. According to their monomeric units, biopolymers are categorized into three parts;

1- Polynucleotide which has nucleotide monomers

2-Polypeptides which are formed by amino acids

3-Polysaccharides which are formed by linear bounded polymeric carbohydrate structures.

Gelatin, chitin, peptides, DNA, RNA are all examples of such biopolymers [7]. Some of the biopolymers are divided in $CO_2$ and water by microorganisms this also means that they are biodegradable [8]. Biodegradable is related with environmentally friendly products that are capable of decomposing back into natural elements.

Biopolymers and their borderline products have diversity and they are crucial and inexhaustible for human life. Their engrossing property increases their importance in medical applications.

This study comprehensively introduces information on special biopolymer such that gelatin and its composition with natural biopolymers like NaCl, RF and glucose also investigates the mechanical properties of gelatin and its several composition with glucose, NaCl and RF in solution form and film form.

## 3. APPLICATION OF BIOCOMPATIBLE MATERIALS

Biocompatible materials shortly known as biomaterials are widely used in modern medical treatments such as artificial tissue, biosensors and drug delivery vehicles. Significant improvements were appeared during the past decades which provide remarkable discoveries in a wide range of diagnostic and therapeutic medical devices [9]. In this study biocompatible polymers were used as chemical in experimental studies so that essentially extraordinary applications of biocompatible polymers were explained during this chapter.

### 3.1. Examples of Biocompatible Polymer Applications

Biocompatible polymers were used to form medical devices or coated onto devices to decrease of rejection by the living organism. Moreover basic qualities of biocompatible materials including comparable strength or hardness to surrounding tissue provide medical treatment to become significant material in tissue regeneration. Generally biocompatible polymers were utilized in medical treatments as implants, membranes for oxygenation and detoxification [10]. Three remarkable improvements that biocompatible materials were used in medical treatment were explained in this part.

#### 3.1.1. Artificial cornea

Artificial cornea is a medical implantation used for transplant patients who could not tolerate a human donor cornea. There are four procedures which are suitable and using in medical treatment: Boston Keratoprosthesis, Osteo-Odonto-Kerastoprosthesis, AlphaCor and Keraklear Artificial Cornea. AlphaCor is the first surgeon in the world which was accomplished in implanting. This surgeon was done by Dr. Ming Wang who entered in the history of medicine with this surgeon [5].

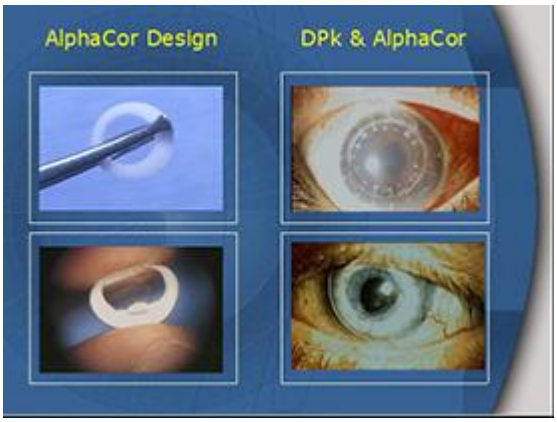



**Figure 3.1:** AlphaCor Keratoprosthesis [Url-6].

AlphaCor is an artificial cornea which is fabricated from biocompatible, flexible, hydrogel material. AlphaCor has two parts one of them is a peripheral skirt and another transparent central region as shown in Figure 3.1. The parts of AlphaCor were connected by an interpenetrating polymer network which was made from poly-2-hydroxyethl methacrylate (pHEMA) [Url-5].

### 3.1.2. Artificial skin

Artificial skin is a synthetic implant which is used generally as human skin in burned patients. Artificial skin is cover material and protects wounded area. Generally skin has two layers, one is called epidermis and another layer is called dermis. The epidermis layer is top layer and dermis places under it. Blood vessels, nerves and hair, oil and sweat glands are in the dermis layer [Url-7].

First synthetic skin was invented by Dr. John F. Burke who is director of the Burn Center at Massachusetts General Hospital and Ioannis V. Yannas chemistry professor at Massachusetts Institute of Technology (MIT) in Cambridge in the early 1970's [Url-7]. Artificial skin firstly was made from polymer which contains collagen fibers and long sugar molecules. This skin was formed by full of small holes material resembling skin. This artificial skin was implanted on the wounds of lab animals. New skin cells are seemed around wounded area. After that these two scientists produced a kind of artificial skin made by polymers from shark cartilage and collagen from cowhide. This new skin was used on a patient who lost over half her body because of burning. After three weeks, patient's new skin emerged at an amazingly healthy rate [Url-8].

Recent time artificial skin was improved using electronic sensors. This new skin quality is less complex and more sensitive. This new skin is flexible skin-attachable strain-gauge sensors which enable to detect pressure, shear and torsion. This new skin is made by polymeric nanofibres which are sustained on thin polydimethylsiloxane layers [11].

### 3.1.3 Gelatin disc-HCEC sheet implantation

Cornea's backside surface is coated by corneal endothelium which is important for regulating and regenerating the injured endothelium. Generally cornea donor was used healing the endothelium. Cultivated cell sheet was improved because of limitations of corneal donor. The basic content of this sheet is gelatin which is used as a carrier substrate. A gelatin carrier includes thermoreversible poly-N-isopropylacrylamide (pNIPAM) culture substrates. Culture of primary human corneal endothelial cells (HCECs) on poly-NIPAM substrates produced an untouched endothelial cell sheet, which was transmitted to a gelatin carrier.

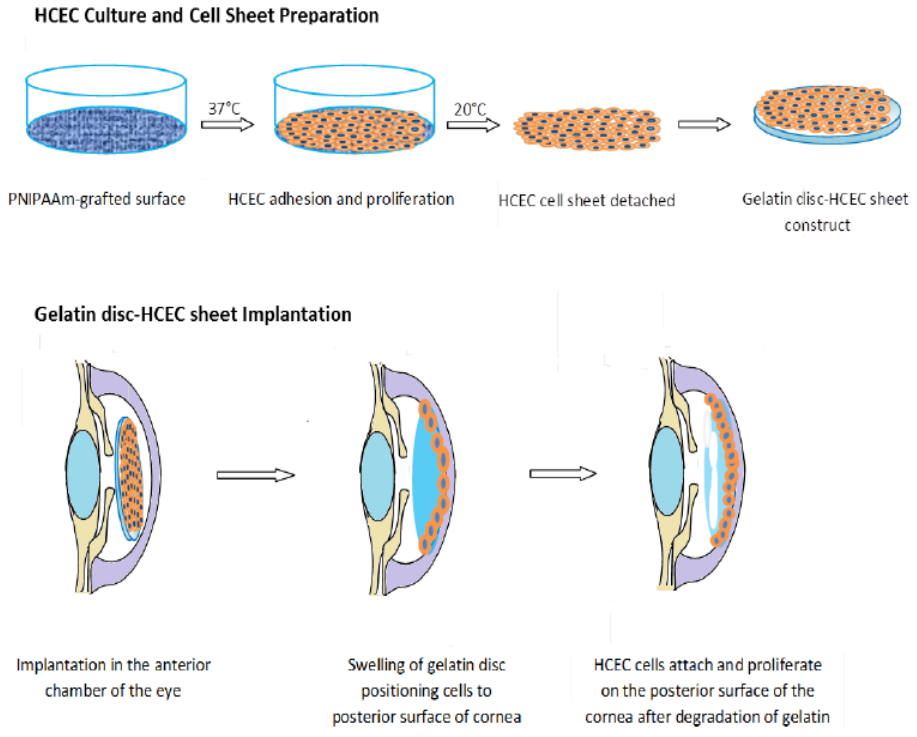



**Figure 3.2:** Schematic diagram of HCEC-gelatin sheet implantation [1].

The gelatin disc enlarged the corneal endothelial cells which play role restoring the cornea to health [1]. In Figure 3.2, a diagram of HCEC-gelatin sheet implantation can be seen as schematically.

## 4. INVESTIGATED PARAMETERS FOR BIOCOMPATIBLE MATERIALS

### 4.1 pH

pH is a numeric scale which indicates the acid potential of aqueous solutions. pH value is important parameter of biocompatibility of a material depending on which sample is desired to be used in many research fields of science.

The symbol "p" means take the negative logarithm of whatever follows in the formula such as pH or pOH. The pH scale is logarithmic scale varies 1 to 14. Mathematically, every multiple of ten in $H^{1+}$ concentration equals one unit on the logarithmic scale. The low pH values indicate a great acid potential while a high pH indicate a low acid potential [12].

"p" is an mathematical operator which evaluates the negative log of any quantity that follows the symbol "p". The definition of pH in equation form is

$$\mathrm{pH} = -\log[\mathrm{H_3O^+}] \tag{4.1}$$

This equation provides the definition of the following series of quantities.

**Table 4.1:** Definitions of special parameters which are used to determine pH [13].

| | |
|---|---|
| pOH = -log[OH-] | the negative log of the hydroxide ion molarity |
| $pK_w$ = -log $K_w$ | the negative log of the water ion product , $K_w$ |
| $pK_a$ = -log $K_a$ | the negative log of the acid dissociation constant, $K_a$ |
| $pK_b$ = -log $K_b$ | the negative log of the base dissociation constant, $K_b$ |

In a water solution the ion product for water is [13]:

$$[\mathrm{H^+}][\mathrm{OH^-}] = \mathrm{K_w} = 1 * 10^{-14} \tag{4.2}$$

Take the -log of both sides of the equation

$$-\log[\mathrm{H^+}] + (-\log[\mathrm{OH^-}] = -\log\,[1 * 10^{-14} \tag{4.3}$$

$$\mathrm{pH} + \mathrm{pOH} = 14 \tag{4.4}$$

pH scale from acidic to alkali and pH value of some body parts were given in Figure4.1.

| Strong acid | | | weak acid | | | **neutral** | weak alkali | | | strong alkali | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | **7** | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| stomach acid | | Vinegar | | skin | | **pure water** | indigestion powders | | washing powder | | | oven cleaner | |

**Figure 4.1:** General pH scale and pH value of some body parts.

### 4.2. Viscosity

Viscosity is the quantity that describes the fluid's resistance to flow shortly. Viscosity is the measure of the internal friction of a moving fluid, if the fluid has large viscosity its molecular form has a lot of internal friction opposite low viscosity fluid moves easily since has a very little friction.

Materials vary from solid to liquid in abstract scale in science, the mechanical properties of solid material is defined as elasticity while liquid material is viscosity. The term of viscosity is related with materials property according to physicist's point of view. This part of physics is known as rheology [Url-9]. Viscosity is measured by viscometer in this thesis rotational viscometer was used in the experimental studies. Viscometer measures ideally viscous fluid and viscosity is defined by two measures; dynamic and kinematic viscosity. Before explaining the dynamic and kinematic viscosity, it is appropriate explaining related terms like shear stress and shear rate.

#### 4.2.1 Shear stress and shear rate

The simple model which is called parallel plate model is used to describe rheological properties. There are two plates which is bonded with each other and filled with fluid in this model. While the lower plate stands, the top plate is moved by a force. The fluid sticks two plates so that does not slide with plate. The flow will be displaced between the plates this is called laminar flow conditions which is main concern for rheological measurements. There is no turbulence flow which affects the rheological properties [Url-10]. Parallel plate model is illustrated in Figure 4.2.

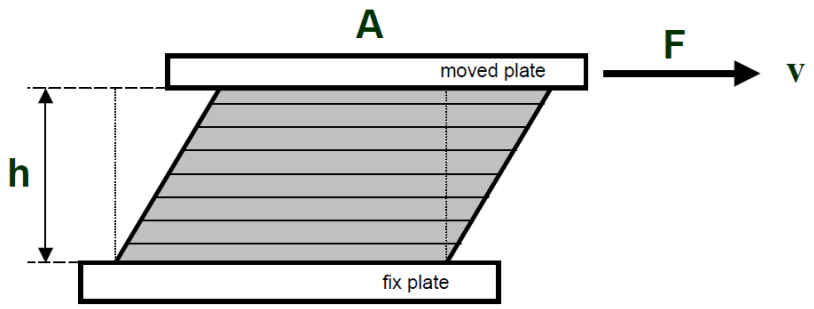



**Figure 4.2:** Parallel plate model [Url-10].

F is the force acting on the top plate and A is the plate's area. The force is measured in Newton, the area in square meters. The distance between the plates is h in term of meter and the v is the velocity of upper plate in term of meter per second.

The shear stress (τ) is defined as the deformation constructed by force F divide into plate's area A. The units of τ are $Nm^{-2}$ or Pa (SI units, $kg*m^{-1}*s^{-2}$); τ may also be expressed in dyne $cm^{-2}$ (c.g.s. units) [14].

$$\tau = \frac{F}{A} = \frac{N}{m^2} = Pa \tag{4.5}$$

10 dyne $cm^{-2}$ = 1 Pa

The shear rate $\dot{\gamma}$ is defined by the change of strain ( $\gamma = \frac{\delta h}{h}$) with time, and has the unit $s^{-1}$.

$$\dot{\gamma} = \frac{\delta \gamma}{\delta t} \tag{4.6}$$

If the flow is linear, a plate at a distance h from the standing plate moving with a velocity v will travel a distance [14]:

$$\delta h = \frac{v}{\delta t} \tag{4.7}$$

$$\dot{\gamma} = \frac{\delta \gamma}{\delta t} = \frac{\delta}{\delta t}\left(\frac{\delta h}{h}\right) = \frac{v}{h} = \frac{ms^{-1}}{m} = s^{-1} \tag{4.8}$$

The shear stress and shear rate values were obtained from rotational viscometer during this thesis. So that physics behind the rotational viscometer is important.

#### 4.2.1.1 Principles of rotational viscometer

Simple rotational viscometer includes motor and measuring unit, user interface, stand, spindle axis, sample-filled cup and measuring spindle (rotor) which was

shown in Figure 4.3. Sample material was dropped into container where measuring spindle was also turned in. Speed of spindle was set to take in consideration of the torque required for turning and then spindle opposite of the fluid's viscous forces is estimated [Url-11].

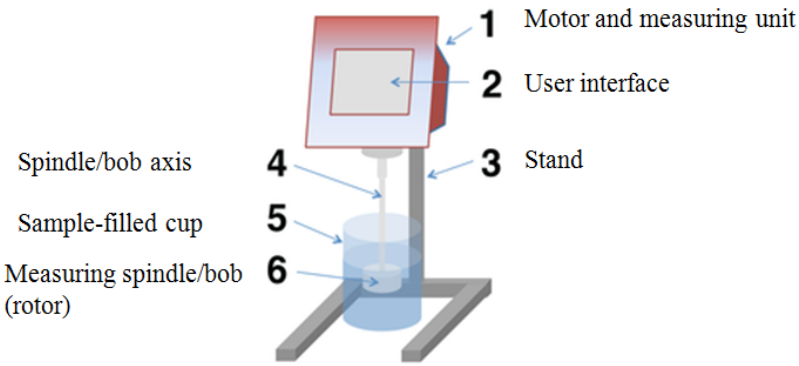



**Figure 4.3:** Main parts of rotational viscometer [Url-11].

Main part of the rotational viscometer is the servo motor which was connected with the measuring spindle directly and demonstrated in Figure 4.4. A high resolution digital encoder on servo motor computes the rotational speed. Viscosity of material is related with torque which is correlated with the motor current. Rotational speed and current are basic parameters to compute viscosity of material [Url-11].

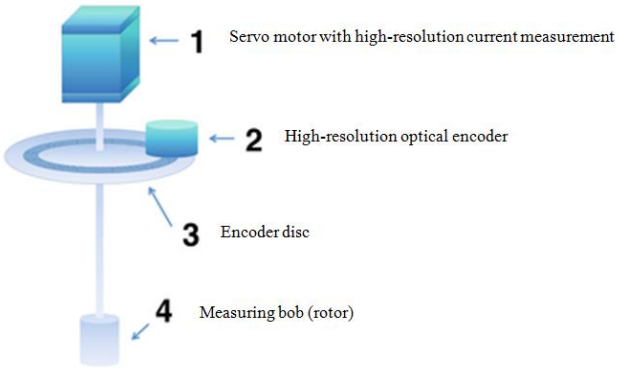
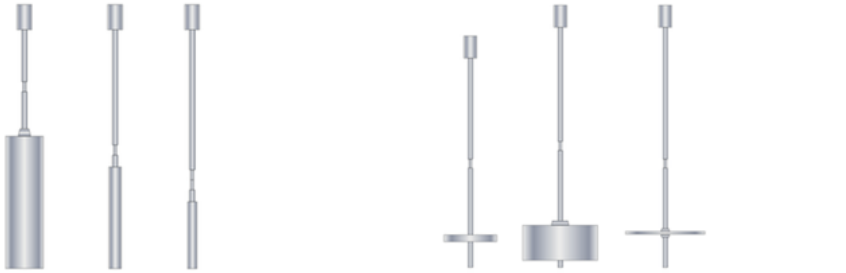



**Figure 4.4:** Rotational viscometer-servo device [Url-11].

Moreover rotational viscometer was adapted several materials using different measuring system such as changing spindle of viscometer. General types of spindles are disc spindles, cylindrical spindles, spindles with spherical shapes and coaxial cylinders shown in Figure 4.5.



a-Cylindrical spindles for rotational viscometers b-Disc spindles for rotational viscometers

**Figure 4.5:** Types of spindles [Url-11].

Viscosity of various type of materials are measured by using different spindles but theoretical evaluation of shear stress and shear rate is similar although spindle shape altered. To obtain dynamic viscosity of material shear stress and shear rate values are determined. Shear stress is correlated with measured torque and geometry of spindles as well as shear rate is calculated from the system's geometry and angular velocity. Coaxial cylinder was used for measuring the viscosity of materials in this thesis. Mathematical equation to evaluate shear stress and shear rate was demonstrated below with its Figure 4.6 [Url-11].

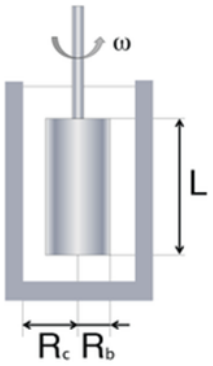



**Figure 4.6:** Coaxial cylinder geometry [Url-11].

$$\dot{\gamma} = \frac{2\,w\,R_c^2}{R_c^2 - R_b^2} \tag{4.9}$$

$$\tau = \frac{M}{2\,\pi R_b^2 L} \tag{4.10}$$

$R_c$ : radius of container (m)

$R_b$ : radius of bob (m)

L : length of spindle (m)

$\dot{\Upsilon}$ : shear rate ($s^{-1}$)

w : angular velocity ($\frac{rad}{s}$)

$\tau$ : shear stress ($\frac{N}{m^2}$)

M : measured torque (Nm)

### 4.2.2 Newton's law and dynamic Viscosity

The main characterization of measurements under steady state is defined by the laws of elasticity (Hooke's law) and viscosity (Newton's law). The dynamic (shear) viscosity of a fluid is related with the fluid resistance to shearing flows which means plates move parallel to each other with dissimilar speeds. Shear stress divided by shear rate is viscosity, which is signed with $\eta$ 'eta'. The viscosity of Newtonian flow is also known as dynamic viscosity which is explained during this part and measured in this thesis.

The stress $\tau$ versus shear rate $\dot{\gamma}$ is linear, and the slope is equal to the viscosity $\eta$ (Pa*s) [14]:

Newton's law:

$$\tau = \eta\dot{\gamma} \tag{4.11}$$

$$\eta = \frac{\tau}{\dot{\gamma}} = \frac{Nm^{-2}}{s^{-1}} = Nm^{-2}s = Pa * s \tag{4.12}$$

1 Pa*s = 10 Poise (P). The viscosity of water at ~ 20 ° C is $10^{-3}$ Pa*s or

1 mPa*s (as this is also $10^{-2}$ Poise or 1 cP, 1 mPa*s = 1 cP).

The result of viscosity in this thesis is typically expressed in centipoises (cP) which is included in c.g.s units.

### 4.2.3 The kinematic viscosity

The kinematic viscosity ($\nu$) is the ratio of the dynamic viscosity ($\eta$) to the density ($\rho$) of the fluid. The unit of kinematic viscosity is the Stoke ( St ) or centi - Stoke ( cSt ), where 1 St = 100 cSt (1 cSt is equal to 1 $mm^2 s^{-1}$ ).

$$\nu = \frac{\eta}{\rho} = \frac{mm^2}{s} = cSt \tag{4.13}$$

The SI unit is square-meters per second [$m^2/s$] or square-millimeters per second [$mm^2/s$]:

$1\ m^2/s = 10^6\ mm^2/s$

Relation between units: 1 cSt = 1 $mm^2/s$ [Url-10].

### 4.2.4 Non - newtonian flow

A non-Newtonian fluid is described as fluid whose viscosity is a function of shear stress or time which can be examined from plots of shear stress τ versus shear rate, as shown in Figure 4.7. Five different flow curves were illustrated: (a) Newtonian; (b) Bingham plastic; (c) Pseudoplastic (shear thinning); (d) Dilatant (shear thickening); and (e) Yield stress and shear thinning. The fluctuation of viscosity with shear rate for the above five systems is shown in Figure 4.8 [14].

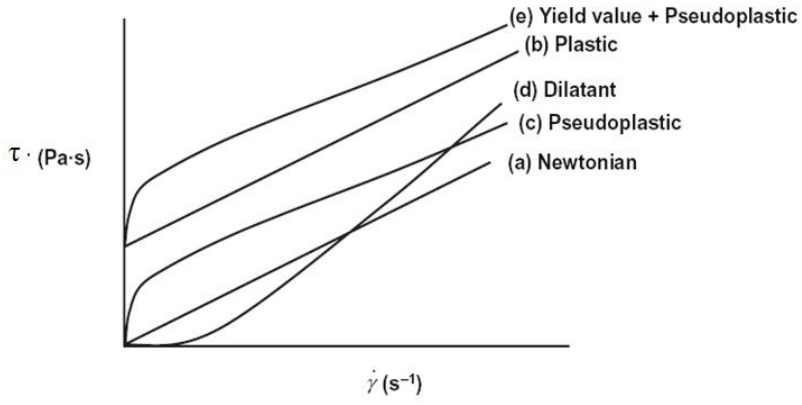



**Figure 4.7:** Flow curves for various systems [14].

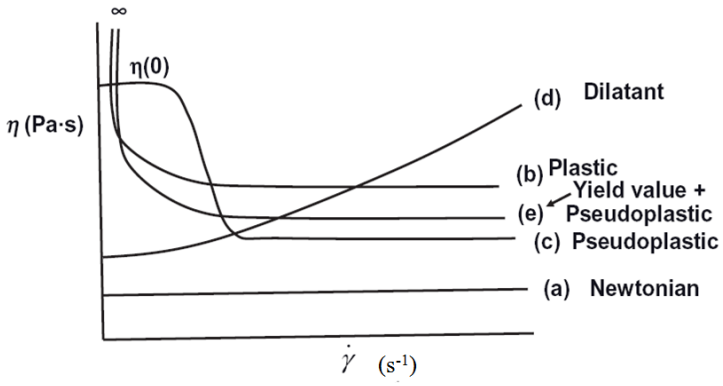



**Figure 4.8:** Viscosity–shear rate relationship for various systems [14].

### 4.3 Rheological Models for the Analysis of Flow Curves

#### 4.3.1 Newtonian systems

In the Newtonian systems, the shear stress linearly related with the shear rate. A Newtonian material referred to as true liquid since the viscosity or consistency did not affect by shear. The flow function of a Newtonian material is a straight line. This line runs through the origin of the coordinate system at an angle α. The relation between shear stress and shear rate is defined as Newton`s law of viscosity [Url-10].

$$\eta = \frac{\tau}{\dot{\gamma}} = \text{constant} \tag{4.14}$$

η is the material constant of the dynamic shear viscosity.

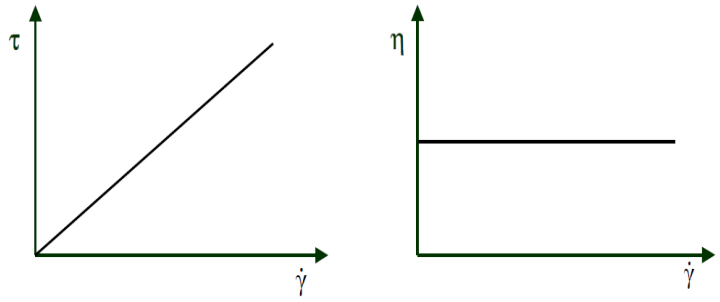



**Figure 4.9:** Flow curve and viscosity curve of Newtonian material [Url-10].

The curves of shear stress versus shear rate and viscosity versus shear rate for Newtonian flow are shown in Figure 4.9.

#### 4.3.2 Pseudoplasticity (shear thining systems)

A shear-thinning or pseudo-plastic fluid is a fluid where viscosity decreases with increasing shear rate. This system does not have a yield value, but rather has a limiting viscosity η at low shear rates.

$$\eta = \frac{\tau}{\dot{\gamma}} \neq \text{constant} \tag{4.15}$$

The proportionality factor $\frac{\tau}{\dot{\gamma}}$ in the Newtonian constitutive equation is thus related with $\eta_a$. $\eta_a$ is the apparent viscosity and shows the viscosity at a certain shear rate $\dot{\gamma}$ [Url-10].

Mathematical expression for pseudoplastic materials according to Power Law fluid model is expressed (Ostwald De Waele) [14]:

$$\tau = k\ \dot{\gamma}^{n} \tag{4.16}$$

Where *k* is consistency index and *n* is the power law index and n<1 for pseudoplastic materials.

Transformed into a viscosity function:

$$\eta = \frac{\tau}{\dot{\gamma}} = k\,\dot{\gamma}^{n-1} \tag{4.17}$$

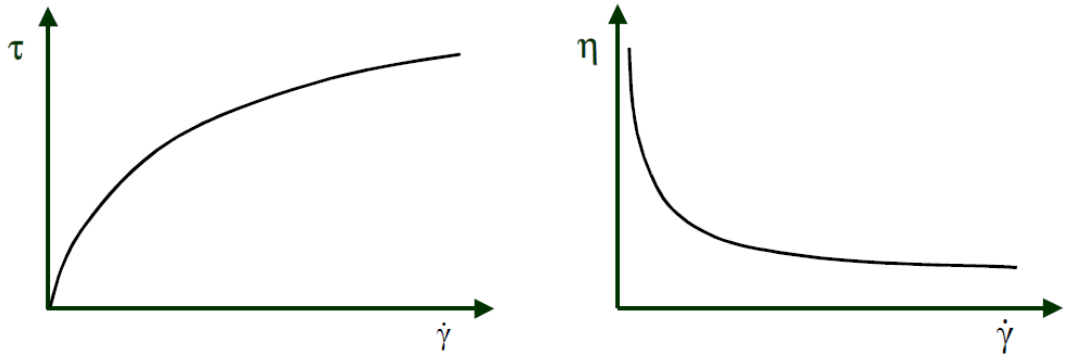



**Figure 4.10:** Flow curve and viscosity curve of pseudoplastic material [Url-10].

Materials are referred to as pseudoplastic such as creams,lotions or gels, if a force acting on the body causes the particle size to change, the particles to be oriented in the direction of flow or an agglomerate to be dissolved.

### 4.3.3 Dilatancy (shear thickening systems)

In the shear thickening system increases the viscosity with agitation or shear strain. Viscosity increases with the shear rate grows.

Mathematical expression of Dilatant materials is defined with the Power Law fluid model (Ostwald De Waele) although *n*>1 [Url-10].

$$\tau = k\,\dot{\gamma}^{n} \tag{4.18}$$

Transformed into a viscosity function:

$$\eta = \frac{\tau}{\dot{\gamma}} = k\,\dot{\gamma}^{n+1} \tag{4.19}$$

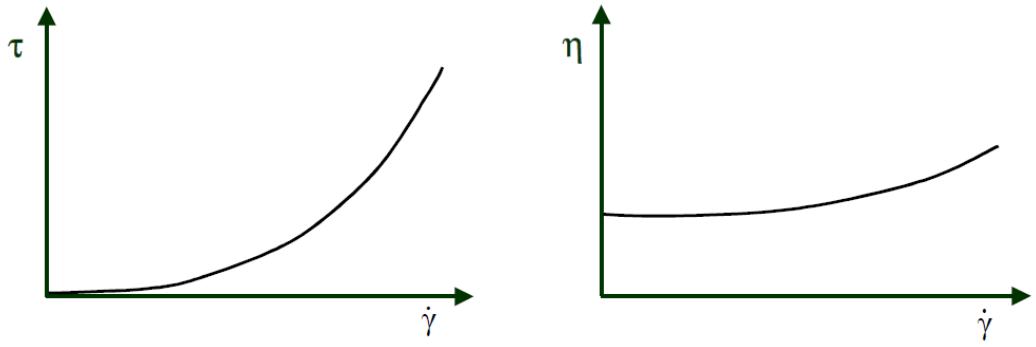



**Figure 4.11:** Flow curve and viscosity curve of dilatant material [Url-10].

Dilatant behaviour can cause trouble in technological processes and is found rather seldom. Wet sand, concentrated corn starch dispersions are examples of dilatant materials.

### 4.3.4 Plasticity and yield point

Plasticity describes structurally viscous liquids which has a yield value $\tau_0$. Plastic material must be exceeded before it will start to flow like a fluid. From that point the viscosity decreases with increasing agitation. Toothpaste, mayonnaise and tomato ketchup are examples of such products. At rest, toothpaste constitutes an network of inter-molecular bonding forces. Material is at rest inter-molecular forces retard the material elements are displaced. If an external force which is smaller than the internal forces or bonding forces acts on the material, the resulting strain is reversible, as with solids oppositely if the external forces exceed the internal bonding forces of the network, the material will start flowing, the solid turns into a liquid [Url-10].

Maximum shear stress $\tau$ at the shear rate $\dot{\gamma} = 0\ s^{-1}$

Thus, if $F_{external} < F_{internal}$, the material does not flow

if $F_{external} > F_{internal}$, the material starts to flow

Plastic liquids flow curve start on the yield point $\tau_0$ , than they converge from the ordinate. The flow curves can be expressed mathematically using a number of equations, depending on the actual material [Url-10].

Mathematical expression according to Bingham:

$$\tau = \tau_B + \eta_B \dot{\gamma} \qquad (4.20)$$

$\tau_B$ is yield point according to Bingham model.

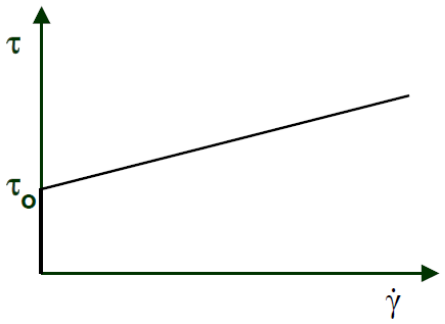



**Figure 4.12:** Flow curve according to Bingham model [Url-10].

Mathematical expression according to Herschel and Bulkley:

$$\tau = \tau_H + k\,\dot{\gamma}^n \qquad (4.21)$$

where n<1 pseudoplastic and n>1 for dilatant materials.

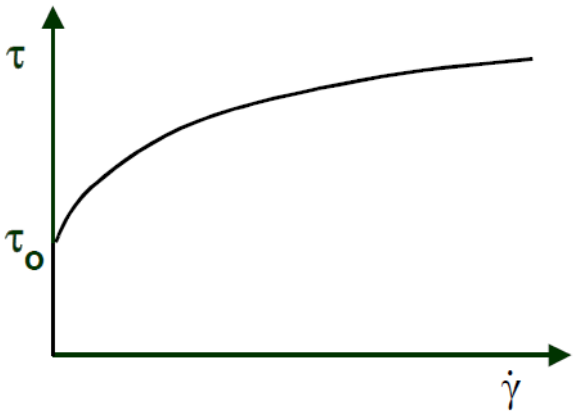



**Figure 4.13:** Flow curve according to Herschel and Bulkley model [Url-10].

Mathematical expression according to Casson [Url-10]:

$$\sqrt{\tau} = \sqrt{\tau_c} + \sqrt{\eta_c \dot{\gamma}} \qquad (4.22)$$

where $\tau_c$ yield point according to Casson model

Physical causes for the occurrence of yield points in dispersions are intermolecular particle-particle and particle-dispersing agent interactions.

### 4.4. Factors Which Affect the Viscosity

External parameters have an impact on the flow and strain behavior of a material. The important parameters are [Url-10]:

- Substance; physical and chemical properties of material such as chemical bonds, polymerization or hardness of material
- Temperature; affect sharply on the viscosity. For example, for liquids viscosity decreases with temperature such as several mineral oils lose about %10 of their viscosity if the temperature is only increased by 1K.
- Time; the viscosity of a material is evaluated on the strain history of a material, in particular on previous loads.
- Pressure; is exerted on a material and then particles are organized in a more tight structure so that when pressure increases the viscosity value decrease
- pH; Viscosity values has linear relation with pH in other words lowest viscosity is observed at the lowest pH, with increasing pH values, the viscosities are observed to increase.

### 4.5 Tensile Test

Tensile test is also called Tension test is a measurement that is an essential in material science to determine material mechanical properties and it is common test to provide basic design information on the strength of materials. Tensile test is an important concept in determining basic mechanical properties of materials beside developing new materials and controlling the quality of materials for use. Material mechanical strength when external forces are loaded and rigidity of material are basic concepts of material mechanical characteristics. Ultimate tensile strength, maximum elongation and reduction in area are measured by this test and Young's modulus, yield strength are determined by analyzing the measurement results that is obtained by stress-strain curve [Url-12].

The test material was prepared suitable for testing machine such that material cross-section is produced in rectangular form and then material was placed in test machine slowly extended until it fractures. During the test, the applied force that produced the elongation on the material was recorded. Load versus elongation or stress versus strain curve was drawn on x-y plane separately. The mechanical behavior of the

material was obtained using these curves besides evaluating parameters which were searched for.

Significant parameters which were evaluated from test result are stress and strain.

Stress (σ); is the force that was acting on material. Mathematically was defined by the magnitude of the force per unit area [Url-13]. The units of stress are $Nm^{-2}$ or Pa (SI units, kg $m^{-1}s^{-2}$) or dyne $cm^{-2}$ (c.g.s. units) [15].

$$\sigma = \frac{F}{A} = \frac{N}{m^2} = Pa \tag{4.23}$$

Strain (ε); the elongation of material that was produced by applied stress and mathematically was defined by elongation over initial length[15].

$$\varepsilon = \frac{\Delta L}{L_0} = \frac{L - L_0}{L_0} = \frac{m}{m} = \text{unitless} \tag{4.24}$$

L: Length after elongation (m) $L_0$: initial length (m)

Stress versus strain curve which is shown in Figure 4.14 was obtained to determine the tensile properties and hence to get valuable information about the mechanical properties of material.

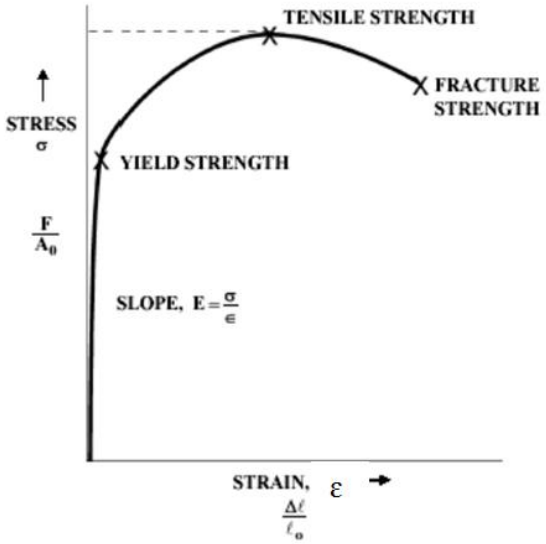



**Figure 4.14:** Stress versus strain curve [Url-12].

There are three types of tensile strength which are shown in Figure 4.14 and explained briefly below [Url-12]:

1-Tensile strength; the maximum stress a material can withstand. Tensile strength is generally used for the purposes of specifications and for quality control of a product. There is also linear correlation between tensile strength and mechanical properties of materials such as hardness.

2-Yield strength; the stress acted on the material at which the material begins to deform plastically. First beginning point of plastic deformation is defined as yielding and it is an essential in material design.

3- Fracture strength; is known as breaking strength and defined by the stress-strain curve at the point of rupture.

Material has a limitation at the tensile strength points. The behavior of material after tensile strength point is explained by the tensile failure in one of two manners:

Ductile failure; extensive plastic deformation in other words necking occurs before fracture.

Brittle failure; plastic deformation does not occur before fracture. Material is suddenly broken in two or more pieces at a low stress state.

### 4.6. Elasticity Modulus

Tensile test is most significant time-independent test in other words stress is applied to material and strain value is obtained. Stress-strain diagram of material explains how material is durable. Analyzing of test results also explain whether material is crosslinked or not depending on increase of the material. Deformation of material and mechanical parameters are obtained from this test. During the test, the machine gives a force that is determined prior to the test (in our test, 500N weight was used) which then the test is performed.

In the initial part of the test, the relationship between the applied force, or load, and the elongation the specimen exhibits is linear. This linear region demonstrates that the ratio of stress to strain is a constant. This relationship is defined by Robert Hooke which is known as “Hooke’s law”. The constant value of stress versus strain ratio is called the “Young’s Modulus” or “Modulus of Elasticity” [16].

The slope of stress versus strain curve in the linear region demonstrates Hooke’s law. Mathematically, Hooke’s law is defined by sequence of equations below [16]:

$$\text{stress } (\sigma) = \frac{\text{force}}{\text{croos-sectional area}} \tag{4.25}$$

$$\text{strain } (\varepsilon) = \frac{\text{change in length}}{\text{real length}} \tag{4.26}$$

In the equations which are given above, the strain has no unit while stress mostly has unit in terms of Pascal (Pa). Using the equations given above, derivation of the mathematical form of stress and strain:

$$\sigma = \frac{F}{A} \tag{4.27}$$

$$\varepsilon = \frac{\Delta L}{L_0} \tag{4.28}$$

where $L_0$ is the real length of the material, A is cross-sectional area, F is applied force to the material and $\Delta L$ is change in length. In the stress versus strain graph, the tangent of the linear region of the curve gives the elasticity modulus, is denoted as E, and the relation between stress and strain becomes:

$$\sigma = \varepsilon E \tag{4.29}$$

In this study, tensile test is applied to the materials since to obtain brief information about tensile testing is given at the rest of this subject. The modulus of elasticity is a measure of the stiffness of the material, but it only applies in the linear region of the curve. If a specimen is loaded within this linear region, the material will return to its exact same condition if the load is removed. At the point that the curve is no longer linear and deviates from the straight-line relationship, Hooke's Law no longer applies and some permanent deformation occurs in the specimen. This point is called the "elastic or proportional limit". From this point on in the tensile test, the material reacts plastically to any further increase in load or stress. It will not return to its original, unstressed condition if the load were removed.

### 4.7. Elastic Potential Energy

Materials deformation such as elongation or compression and fracture by external forces were defined as mechanical properties of materials. If external forces were applied to the mechanical systems, deformation was occurred and elastic potential energy was stored under the condition of compressing and stretching of systems.

Main concern about elastic potential energy is reversibility. That is, potential energy was stored into material if original shape of material could be recovered and there was not any limitation for yielding point of material. This limit was also called elastic limit was related with materials degree of distortion which could be tolerated without breaking and irreversibility altering their internal structure. Materials could not store energy as the form of elastic potential energy above the elastic limit. Therefore elastic potential energy provides to predict many mechanical properties such as elasticity and strength of materials. Materials were chosen for a specific purpose under favour of materials performance at different conditions and after that analyzing stress-strain curve [17].

Mechanical properties of material mathematically were described by Hooke's law. According to Hooke's law stress-strain relation was expressed by Equation 4.29 where E is the modulus of elasticity or Young`s modulus.

Moreover it is appropriate to write Hooke`s law using the terms of different cross-sectional are $A_0$, different length $L_0$ and force F as [Url-13];

$$\frac{F}{A_0} = E\frac{\Delta L}{L_0} \tag{4.30}$$

In the elastic limit modulus of elasticity E is the slope of the stress $\frac{F}{A_0}$ versus strain$\frac{\Delta L}{L_0}$.

Hooke's law was returned to

$$F = \frac{EA_0}{L_0}\Delta L = kx \tag{4.31}$$

This equation also describes the stiffness of an ideal spring, where it comes in saturation $k = \frac{EA_0}{L_0}$ and $x = \Delta L$.

The elastic potential energy stored is given by the integral of Hooke`s law expression with respect to ΔL

$$U_e = \int \frac{E\,\Delta L\,A_0}{L_0}\,d\Delta L = \frac{EA_0}{L_0}\int \Delta L\,d\Delta L = \frac{E\,A_0\,\Delta L^2}{2L_0} \tag{4.32}$$

The elastic potential energy per unit volume is given by

$$\frac{U_e}{A_0 L_0} = \frac{E\,\Delta L^2}{2L_0^2} = \frac{1}{2} E\varepsilon^2 \tag{4.33}$$

The elastic potential energy formula can also be expressed as the integral of Hooke`s law [17].

$$U_e = \int kx dx = \frac{1}{2}\, kx^2 \tag{4.34}$$

The characterizations of materials include specification, usually in terms of strains, of its elastic limits. Beyond the elastic limit, a material is no longer storing all of the energy from mechanical work performed on it in the form of elastic energy.

## 5. BIOMATERIALS USED IN EXPERIMENTAL STUDY

### 5.1. Gelatin

Gelatin is a biocompatible material which can be sourced by collagen such as hides, bones and tendons. Generally, in mammals one third of total body protein consist of collagen molecule whose sub-unit is known as tropocollagen. There are two basic types of gelatin; one of them is constructed acid extracted gelatin which is called type A. On the other hand type A is formed through acidic treatment prior to thermal application. Another type of gelatin is called type B and it is formed by alkaline treatment in order to comprise the several of carboxylic compounds. Moreover, scientific researches and experiments about collagen structures relying on gelatin manufacture introduces that type of collagen and the age of the animal can impact the properties of gelatin and its structure which is illustrated in Figure 5.1[Url-14].

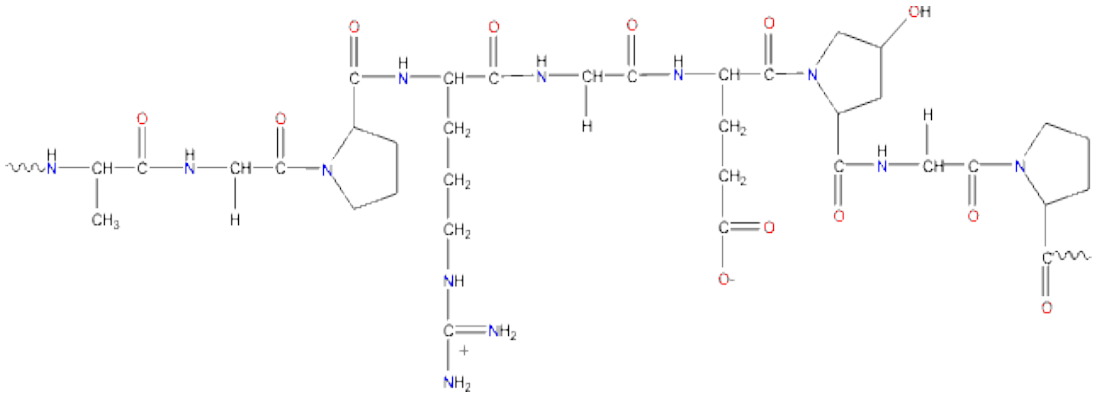


**Figure 5.1:** Chemical structure of gelatin [Url-15].

Gelatin is obtained from collagen and it is formed by 50.5% carbons, 6.8% hydrogen, 17% nitrogen and 25.2% oxygen. Gelatin is produced at the end of protein reactions. Gelatin has composition of peptide and amino acid components. Gelatin is also soluble in water to about $40^0$C approximately 30 minutes. Solution form of gelatin which is known as gelatinous solution can be varied to bio-gels or films under suitable conditions. Generally gel form of gelatin for many applications is obtained

by cooling gelatin below approximately $35^0$C. Gelatin gel strength and viscosity is correlated with its molecular weight in other words, low molecular weight means low gel strength and viscosity. Another important parameter of gelatin is its pH value which is also crucial for biocompatibility. pH value of gelatinous solution is approximately 5.[Url-14]

Applied temperature is playing an important role to produce the form of gels and films during the experimental process. The form of films and gels of gelatin is widely used in tissue engineering and biomedical applications as treatment or solution. As an experimental result due to the weakness of the form of gels of gelatin, the numerous of solutions have been carried out so as to make the material more durable from mechanical aspect [18].

### 5.2 Riboflavin

Riboflavin is generally known as vitamin B2, is type of a biocompatible chemical compound that consists of oxygen, hydrogen atoms and hydro oxide groups and provides to start photoreactions in solutions [Url-16]. Sensitivity of the material is mostly depending on environmental conditions, pH and external factors. Applications of the light, in particular carrying out of UV light on riboflavin ensure the excitation in atomic levels of its molecular structure at the room temperature. Degradation of the material can usually be observed at greater than $37^0$C experimentally. The general chemical structure of riboflavin is given in Figure 5.2. The chemical structure of riboflavin includes vary of 6 tetrahedron, 3 benzene rings and chemical bonds. Riboflavin is also an effective water soluble compound as its structure is polar and can easily be reacted with gelatin in the state of solution [Url-17].

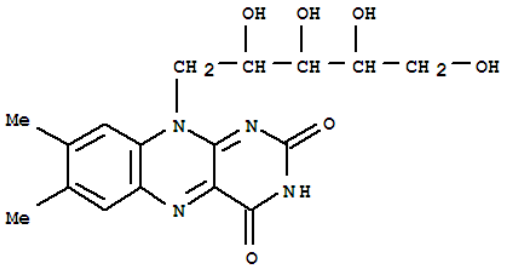



**Figure 5.2:** Chemical structure of riboflavin [Url-18].

The chemical formula of riboflavin is symbolized as $C_{17}H_{20}N_4O_6$ and has a molecular weight as 376.2051 gram per mole approximately [Url-17].

One of the important applications of riboflavin is to be used in medicine as a treatment, especially in ocular disorders such as kerataconus disease.

### 5.3 Glucose

Glucose is a simple sugar also called as monosaccharide which is a carbohydrate and crucial for human metabolism. Glucose is the basic molecule which is directly absorbed into the blood stream during digestion in order to serve energy to organism [Url-19]. Moreover, glucose is basic products of photosynthesis and it is energy source of cell respiration [Url-20]. Glucose is a also polymer molecule which is stored as starch in plants and glycogen in animals [Url-21]. Glucose molecule is signed by molecular formula $C_6H_{12}O_6$ and its molecular structure is illustrated in Figure 5.3.

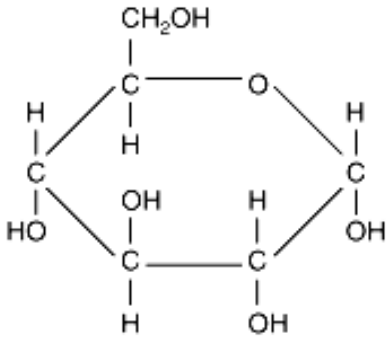



**Figure 5.3:** Molecular structure of glucose [Url-22].

Glucose molecule alters mechanical properties of gelatin solution. If concentration of glucose increases, strength of gelatin solution decreases. In other words, glucose molecule has plasticizing effect on gelatin solution because of competition of glucose molecule for usable water.

### 5.4 Salt (NaCl)

Salt is an inorganic compound which is formed by the electrostatic attraction between negatively charged ion which is called anion and positively charged ion which is called cation. Moreover, this electrostatic attraction between ionic

compounds produces an ionic bond that is basic to maintain the chemical structure of salt [Url-23; 24].

NaCl which is formed by positive sodium ion and negative chloride ion is most important salt for nature and biological systems. Chloride ion is attracted by sodium ion and ionic bond is formed. Physical appearance of NaCl is a solid, clear crystal and it is soluble in water. The crystal structure of NaCl that has regular and repeating structure is demonstrated in Figure 5.4

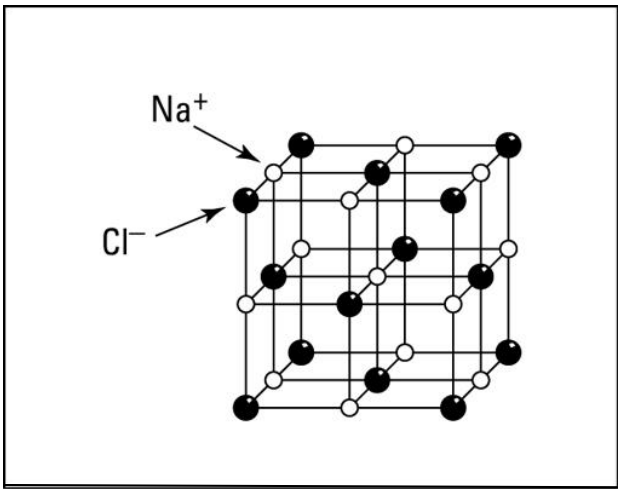



**Figure 5.4:** Crystal structure of sodium chloride [Url-25].

Structure of NaCl is formed as molecules stacking on top of each other which is defined as lattice and solid form of NaCl generally contains lattice-type arrangement [Url-23;24].

# 6. EXPERIMENTAL PROCEDURE

## 6.1 Preparation of Solutions

In the academic literature, there are plenty of rheological studies related to viscosity measurements of collagen gels and hydro-gels, particularly gelatin and other natural polymers so that gelatin (Doga Drug-Gelatin Powder Bloom) was chosen as a based material. First of all to create any convenient concentration, various concentrations of gelatin were prepared. After that, other natural polymers such as riboflavin (Sigma Aldrich, ≥98% purity), glucose (Merck KGaA, M=180.16 g/mol) and salt (Sigma Aldrich, ≥ 98% purity) were added to determined concentrations of pure gelatins which were used as based materials during this study. The pure gelatin was 80% and other components were added as 20% separately. Each solution was prepared under the same conditions such that laboratory temperature was always controlled at $24^0$C. Ingredients of each solution were measured by same equipment such that all materials were weighed by XB220A type Presca balance and a magnetic little mixer was preferred to provide a better and a quick solution that includes all the substances were dissolved clearly.

### 6.1.1 Preparation of pure gelatin solutions

In order to obtain suitable concentration of gelatin, six different concentrations were selected which was demonstrated in Table 6.1. Each solution was prepared under the same conditions and totally 30 g. Procedures for preparation of pure gelatin solutions were clarified below:

**Table 6.1:** Gelatin amounts for corresponding ratio.

| Proportion $(\frac{\text{solute}}{\text{solvent}})$ | 5% | 3.3% | 2% | 1.42% | 1% | 0.6% |
|---|---|---|---|---|---|---|
| Gelatin(g) | 1.5 | 1 | 0.6 | 0.439 | 0.3 | 0.2 |
| $dH_2O$(ml) | 28.5 | 29 | 29.4 | 29.571 | 29.7 | 29.8 |

5% pure gelatin solution contains 28.5 ml distilled water and 1.5 g gelatin. 1.5 g gelatin was dropped into distilled water slowly when solution was stirred on magnetic mixer which was set 1200 rpm in order to mix the solution by the help of magnetic fishes on the heater at $80^0$C. After a half an hour, the mixture process has been completed. Afterwards, the solution was cooled at the room temperature ($24^0$ C) in order to measure the pH and viscosity of solution.

Procedure which was explained above for 5% were applied to each concentration such as 3.3%, 2%, 1.42%, 1% and 0.6% one by one according to the Table 6.1.

5% and 2% concentrations of gelatin were determined as a base material. Because of the fact that the 5% gelatin concentration is most intensive one and 2% gelatin concentration has suitable mechanical characteristic as solution form and film form.

### 6.1.2 Preparation of gelatin based riboflavin solution

To investigate the effect of riboflavin and UV light on mechanical properties of gelatin, riboflavin was added to pure gelatin solution. To prepare pure gelatin solutions as based materials the same procedures which were explained in previous part were applied as mentioned above.

Apart from gelatin, the riboflavin was prepared sensitively because of altering the properties of riboflavin depending on light and the temperature. Effect of UV sensitive riboflavin was investigated before UV light and after UV light so that, the roller blinds and the lights were turned off and the glass beaker was coated with the aluminum in order to prevent the specimen from the light.

The riboflavin was prepared at the portion of 0.05% and totally 6 grams. 0.003 g riboflavin and 5.97 ml distilled water were measured for preparing the 0.05% riboflavin solution. Ingredients were mixed by the help of magnetic fish on heater at $37^0$C as riboflavin is disrupted above this temperature and magnetic mixer was set 1000 rpm in order to dissolve completely. 80% of the gelatin based riboflavin solution was obtained from gelatin solution and 20% of gelatin based riboflavin solution was obtained from the riboflavin solution. These pure gelatin solution and riboflavin solution were mixed at $24^0$C half hour in order to mix each other completely. After that the pH and viscosity measurements were done for both 2% and 5% gelatin based-riboflavin solutions before UV light and after UV light which was 370 nm approximately 2 hours. Besides the investigating the affect of UV,

influence of time interval on gelatin-based riboflavin solution was analyzed. In order to analyzing the influence of time interval four 2% gelatin-based riboflavin solutions were prepared with the similar procedures as mentioned above. Afterwards 2% gelatin-based riboflavin solutions were exposed to UV light at 370 nm at different time intervals such those 15 minutes, 30 minutes, 45 minutes and 60 minutes one by one. Solutions were stirred at constant speed during radiation.

### 6.1.3 Preparation of gelatin based glucose solutions

Glucose molecule in gelatin solution mostly influences the mechanical strength of gelatin because of contention of the sugar for convenient water as well as a general plasticizing effect of sugar on gelatin so that several concentrations of glucose which was shown in Table 6.2 were prepared and mixed with 5% and 2% gelatin solutions one by one. 5% and 2% gelatin solutions were selected base solutions and prepared with similar procedure as mentioned before.

**Table 6.2:** Glucose amounts for corresponding ratio.

| Proportion $(\frac{\text{solute}}{\text{solvent}})$ | 1% | 2% | 3% | 4% | 5% |
|---|---|---|---|---|---|
| Glucose(g) | 0.06 | 0.12 | 0.18 | 0.24 | 0.3 |
| $dH_2O$(ml) | 5.94 | 5.88 | 5.82 | 5.76 | 5.7 |

Each glucose solution was prepared total 6 g with different concentrations of glucose which was demonstrated in above.

In order to prepare 1% glucose concentration, 0.06 g glucose was dropped into 5.94 ml distilled water slowly when solution was stirred on magnetic mixer which was set 1000 rpm because of mixing the solution by the help of magnetic fishes on the heater at $24^0$C. After 20 minutes, the mixture process of glucose was completed.

Procedure which was explained above for 1% were applied to each concentration such as 2%, 3%, 4% and 5% one by one according to the Table 6.2.

Afterwards, for each glucose concentration, 20% of gelatin based glucose solution was formed from glucose solution and 80% of gelatin based glucose solution was from 5% and 2% gelatin solutions separately. Pure gelatin and glucose solution were mixed at $24^0$C half hour in order to mix each other completely. After that the pH and

viscosity measurements were done for both 2% and 5% gelatin based-glucose solutions.

### 6.1.4 Preparation of gelatin based NaCl solutions

Effect of NaCl on gelatin was investigated in last part so that several concentrations of NaCl which was shown in Table 6.3 were prepared and mixed with 5% and 2% gelatin solutions one by one. 5% and 2% gelatin solutions were selected base solutions and prepared with similar procedure as mentioned before.

**Table 6.3:** NaCl amounts for corresponding ratio.

| Proportion $(\frac{\text{solute}}{\text{solvent}})$ | 1% | 2% | 3% | 4% | 5% |
|---|---|---|---|---|---|
| NaCl(g) | 0.06 | 0.12 | 0.18 | 0.24 | 0.3 |
| $dH_2O$(ml) | 5.94 | 5.88 | 5.82 | 5.76 | 5.7 |

Each NaCl solution was prepared total 6 g with different concentrations of NaCl which was demonstrated on table above. In order to prepare 1% NaCl concentration, 0.06 g NaCl was dropped into 5.94 ml distilled water slowly when solution was stirred on magnetic mixer which was set 1000 rpm to mix the solution by the help of magnetic fishes on the heater at $24^0$C. After 20 minutes, the mixture process of NaCl was completed. Other concentrations of NaCl such that 2%, 3%, 4% and 5% were prepared one by one with the similar procedure like 1%.

After preparation of each NaCl solutions separately, 5% and 2% pure gelatin solutions were taken at the 80% and each NaCl solutions were taken 20% and mixed together due to prepare gelatin based-NaCl solutions. Pure gelatin and NaCl solution were mixed at $24^0$C half hour in order to mix each other completely. After that the pH and viscosity measurements were done for both 2% and 5% gelatin based- NaCl solutions.

## 6.2 pH Measurement of Gelatin Based Solutions

One of the most important parameter of controlling the solution whether is acidic or not can be defined as pH. Measurement of pH can also be considered as a parameter of biocompatibility of a material depending on which sample is desired to be used in many research fields of a science. pH level measurements mechanism is the

combination of a HI2221 Calibration Check pH/ORP Meter from Hanna Instruments and a computer. The pH meter is connected to computer with a USB cable and it has an adjustable handle carrying the tip which measures the pH level of the solutions. In Figure 6.1 the instrument is shown below.

**Figure 6.1:** pH meter using during the measurements.

Respectively before viscosity measurements of solutions, the tip of the mechanism is plunged into the solution cup and the value of the pH was read from the screen of the instrument. All pH data were saved by hand which are shown in Table 6.4.

**Table 6.4:** pH level of all solutions.

| Solution | pH |
|---|---|
| 5%pure gelatin | 5.54 |
| 3.3%pure gelatin | 5.87 |
| 2% pure gelatin | 5.59 |
| 1.42%puregelatin | 5.69 |
| 1% pure gelatin | 5.73 |
| 0.6% pure gelatin | 5.87 |
| 2% +RF Before UV | 5.63 |
| 2% G+RF After 15 min. UV | 6.63 |
| 2% G+RF After 30 min. UV | 5.63 |
| 2% G+RF After 45 min. UV | 5.63 |
| 2% G+RF After 60 min. UV | 5.63 |
| 5% G+1% Glucose | 6.50 |
| 5% G+2% Glucose | 6.50 |
| 5% G+3% Glucose | 6.48 |
| 5% G+4% Glucose | 6.38 |
| 5% G+5% Glucose | 6.36 |
| 5% G+1% NaCl | 6.35 |
| 5% G+2% NaCl | 6.24 |
| 5% G+3% NaCl | 6.19 |
| 5% G+4% NaCl | 6.17 |
| 5% G+5% NaCl | 6.14 |
| 2% G+1% Glucose | 6.18 |
| 2% G+2% Glucose | 6.16 |
| 2% G+3% Glucose | 6.14 |
| 2% G+4% Glucose | 6.06 |
| 2% G+5% Glucose | 6.04 |
| 2% G+1% NaCl | 6.02 |
| 2% G+2% NaCl | 6 |
| 2% G+3% NaCl | 5.97 |
| 2% G+4% NaCl | 5.96 |
| 2% G+5% NaCl | 5.96 |

pH level of all solutions alter 5.54 to 6.63 as shown in Table 6.4. The pH level of cornea is 7.44±0.22 and pH tolerance of cornea is 3 to 10 and human skin pH level is 5.4 to 5.9. This means that all solutions obey the biocompatible sensitivity with option considered pH.

### 6.3. Viscosity Measurement of Gelatin Based Solutions

In this thesis, the viscosity of each solution was analyzed one by one. These analyzes involve the preparation and measurement of each solution under the standard conditions such that viscosity measurements of each solution were done at $24^0$C room temperature. Pure gelatin solutions and gelatinous solutions were defined by their shear stress versus shear rate and viscosity values. The viscosity is a measure of the resistance to gradual deformation by shear stress that is mentioned before. The low viscosity measurements were performed using the Fungilab Rotational Viscometer. The figure 6.2 below shows the Fungilab Rotational Viscometer that is used for testing in this project.


**Figure 6.2:** Fungilab Rotational Viscometer (For viscosity measurement).

Generally sample solution was dropped into container where measuring spindle was also turned in. Speed of spindle was set to take in consideration of the torque required for turning and then spindle opposite of the fluid's viscous forces is estimated. Data which include shear stress, shear rate, viscosity, torque and speed were saved in computer as special software program.

There are generally two types of simple flow characterization tests for viscometer. These are stepped shear rate (=rotational speed) and constant shear rate.

In stepped shear rate, individual shear rate values are selected. In this thesis shear rate values are determined according to solution concentration between at least10 rpm to at most 220 rpm for rising and 220 rpm to 10 rpm for declining at expansion ratio of 10 rpm . Each shear rate is applied at 90 second and then shear rate, shear stress and viscosity are recorded for each value. The results of measurements are illustrated by shear stress versus shear rate graphs for each solution in last part.

### 6.4 Preparation of Films

Pure gelatin films and gelatinous films were prepared using the pure gelatin, gelatin based-riboflavin, gelatin based-glucose and gelatin based-NaCl solutions. Therefore preparing of each solution was not explained again in this part. Each gelatin and gelatinous solutions were turned to film at $24^0$C by using PEG slides. Every solution was cooled $24^0$C and dropped on PEG slides separately afterwards each of them was dried at least 48 hours in close environment to obtain films.

### 6.5 Tensile Test of Films

INSTRON 3345 tensile test machine which is shown in Figure 6.3 was performed to determine tensile strength, tensile stress and elongation at break for each pure gelatin films and composite films, respectively.

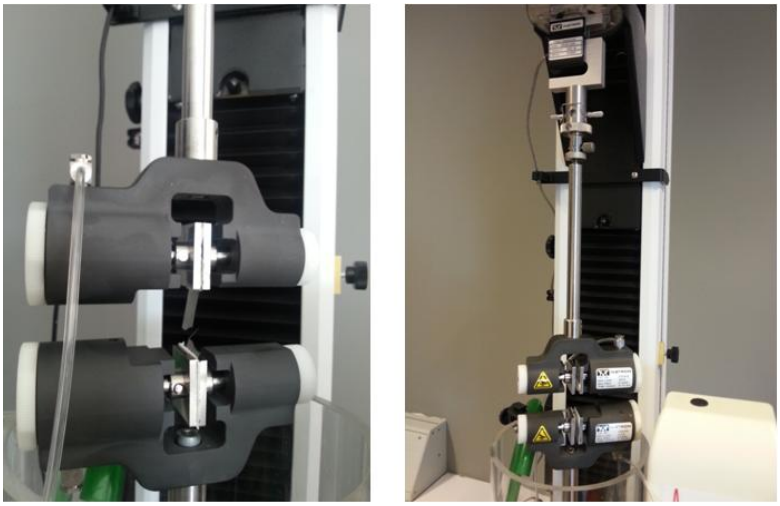


**Figure 6.3:** INSTRON 3345 tensile test machine.

The tensile testing machine, set at crosshead speed of 0.05 mm/s, and load cell of 500 N sensibility were used to perform uniaxial tensile. Prior to testing, the thickness

of each film was measured with a Micrometer Gauge (with 0.001 precision). Using the Instron instrument, an axial tensile test was run on each film. Samples that had air bubbles and/or tears were not tested. All tests were performed under ambient conditions such as at $24^0$C room temperature.

The output data of the Instron instrument for tensile testing included data for the true stress and true strain recorded for each film during testing. The true stress and true strain data was used to produce a stress-strain graph for each sample.The stress-strain graph of each sample begins with a region with ~ 0 slopes. It then transitions to a constant slope region; the slope of this region is the Modulus of Elasticity of the film.

# 7. RESULTS AND DISCUSSION

## 7.1 Flow Behavior of Solutions

Measuring viscosity of solution is important in order to analyze solution mechanical characteristic and predict solution behavior under external factors such as external force. Moreover, viscosity measurement provides advantages to control production processes such that external factors acting on product performance can be understood most accurate and reliable way with this measurement. On the other hand, viscosity measurement contributes the research of chemical, mechanical ant thermal treatments and the impacts of contributed materials.

Another step besides the viscosity measurement was to obtain data from viscosity measurement in order to determine flow behavior of the solution. Flow behavior of solution which was obtained after viscosity measurement demonstrates viscosity values and solution type such as Dilatant or Pseudoplastic according to the relation between shear stress and shear rate. Shear stresses versus shear rate data were used for graphic demonstration. Shear stress values were placed on the ordinate as well as shear rate on the abscissa. Shear stress versus shear rate graphic demonstration indicates flow function or flow curve which was used to characterize solution type with respect to mathematical model.

As a result of these reasons which were explained above the flow curve of pure gelatin solutions, gelatin based-riboflavin solutions, gelatin based-glucose solutions and gelatin based NaCl solutions were illustrated in Figure 7.1 to 7.8.

The flow behaviors of pure gelatin concentrations percentages of 5% and 3.3% which were most intensive solutions and could be measured at lower speed than other concentrations was illustrated in Figure 7.1.

According to flow behavior Figure 7.1, these solutions could be characterized as non-Newtonian solutions because of their flow lines starting with yield point. In

other words, the flow curves of both solutions demonstrated that the measured inclining and declining curves did not coincide. The gap between curves was called hysteresis area. On the other hand, magnitude of the time-dependent flow behavior was identified by hysteresis area which was defined as thixotropy. Thixotropy was characteristic of non-Newtonian solution which was obtained as a result of decreasing the viscosity of solution with increasing time of shearing. These both pure gelatin solutions acted as thixotropic materials according to their flow behaviors. The plasticize of these two solutions was occurred for several reasons such that rod-like particles array in the flow direction, lacking of joints in polymer solutions, coordination of microstructure in suspension and emulsion flow and breakdown of aggregations [19]. Inflection on the margin of 3.3% gelatin concentration flow was occured as a result of digression measuring range.

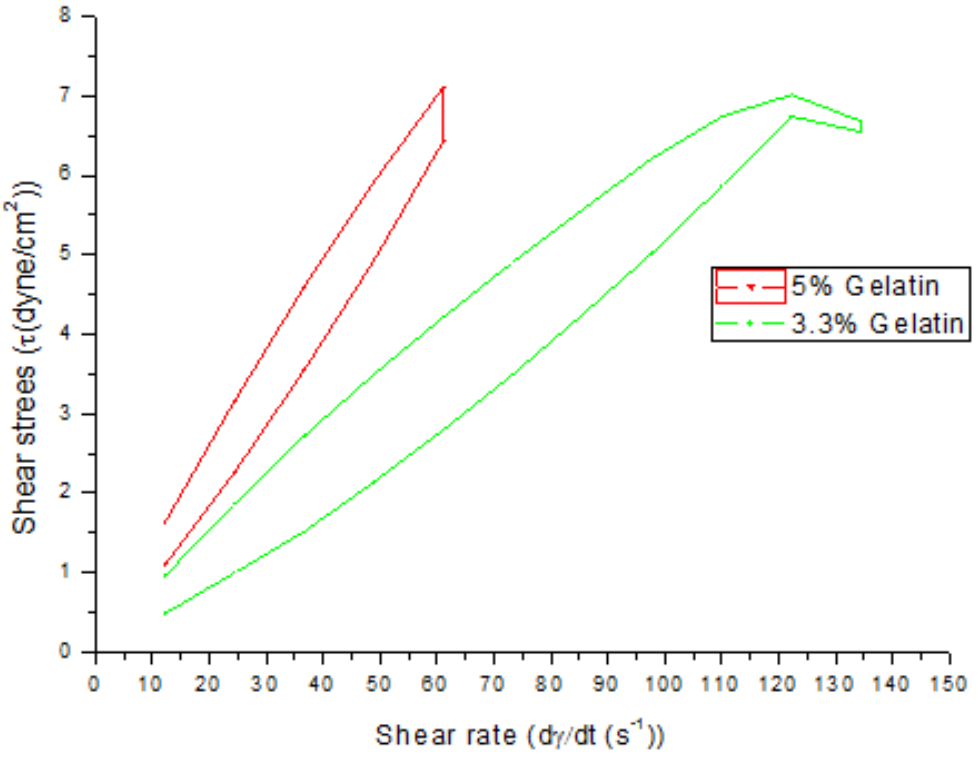



**Figure 7.1:** Shear stress versus shear rate graph for 5% and 3.3% pure gelatin solutions.

The flow behaviors of other concentrations such as 2%, 1.42%, 1% and 0.6% were characterized in Figure 7.2. Only 2% pure gelatin solution was defined as Newtonian fluid compare with flow diagrams of each solution.

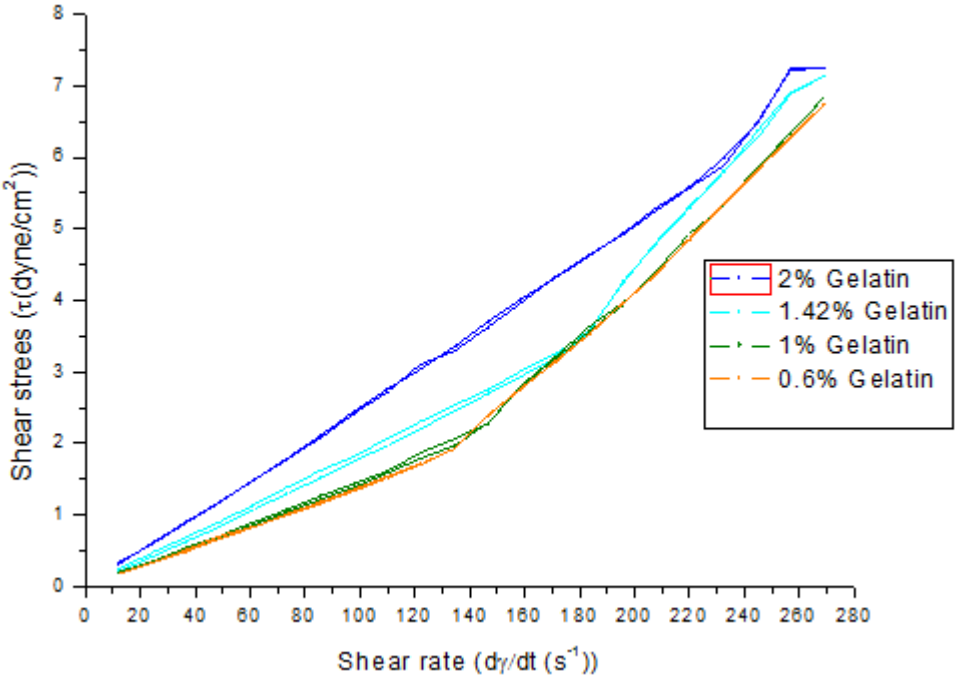



**Figure 7.2:** Shear stress versus shear rate graph for 2%, 1.42%, 1% and 0.6% pure gelatin solutions.

1.42%, 1% and 0.6% pure gelatin solutions indicated Dilatant or shear thickening behavior. Dilatant fluids like these three gelatin solutions are non-Newtonian fluids, it means that these solutions were not demonstrated a linear shear stress versus shear rate. Dilatant behavior was influenced on particle size, shape and distribution. Moreover, this behavior was happened as a result of colloidal suspension transitions from a stable state of flocculation. The characteristics of suspensions were attached to Hamaker theory and Van der Waals forces. These suspensions were balanced electrostatically or sterically [Url-26]. These three gelatin solutions like most of dilatant materials behaved like a fluid under the conditions of low shear forces and then transverse solid-like behavior when shear forces increased. Besides the shifting of molecules from a layered to a disorder structure, viscosity of Dilatant material was increased as a result of transition from low shear forces to high shear forces which were higher than repulsive interparticle forces.

The shear stress-shear rate curves of gelatin riboflavin solutions at before and after UV are shown in Figure 7.3. Two different concentrations of gelatin are chosen such that 5% and 2%. Riboflavin was added both concentrations and viscosity

measurement were taken for both of them after and before 370 nm UV light at 1 hour.

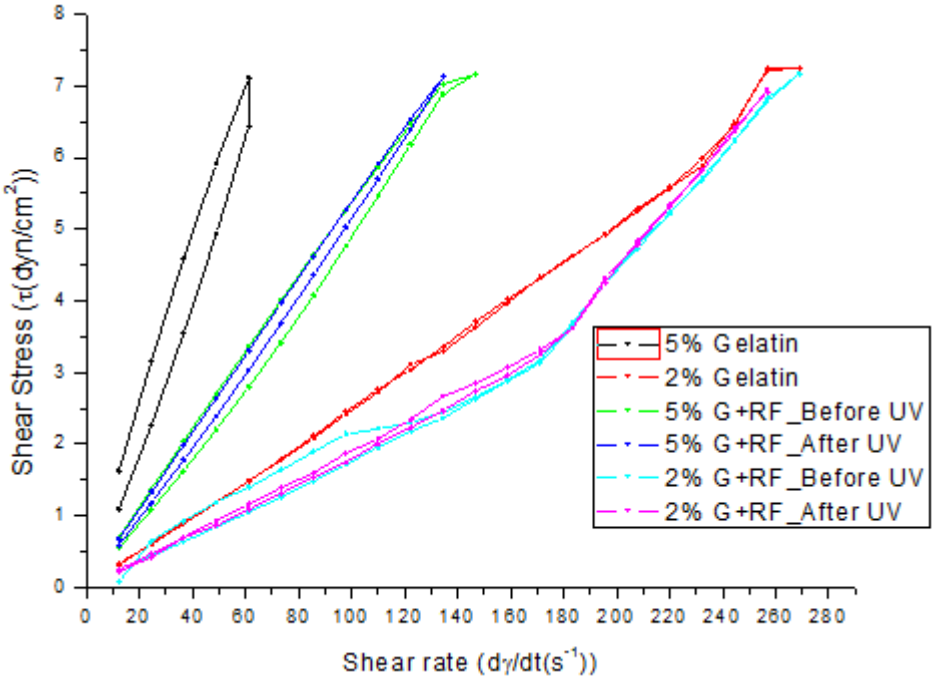



**Figure 7.3:** Shear stress versus shear rate graph of 5% and 2% gelatin and gelatin based-riboflavin solutions.

The Figure 7.3 illustrated that the gap between inclining and declining curves became narrow; it means that thixotropy property of material was decreased as a result of adding riboflavin and UV radiation which is known as photoradiation. Moreover, yield point of 5% pure gelatin solution decreased by the influence of riboflavin and photoradiation. In other words, external forces went over the limit of the internal bonding forces of 5% gelatin based riboflavin solution before and after UV, then solutions started to flow easily than its pure form. The 2% pure gelatin solution varied Newtonian to non-Newtonian flow behavior. Adding riboflavin and photoradiation affected viscosity of pure form which turned to Dilatant or shear thickening behavior. 2% pure gelatin solution translated to colloid form as a result of riboflavin and photoradiation factors caused to disordered structure.

The affect of UV absorbance time was investigated by using the 2% pure gelatin based-riboflavin solutions. 370 nm UV light was applied to 2% gelatin based-riboflavin solution at 15 minutes, 30 minutes, 45 minutes and 60 minutes. Figure 7.4 illustrated shear stress shear rate curves of these solutions.

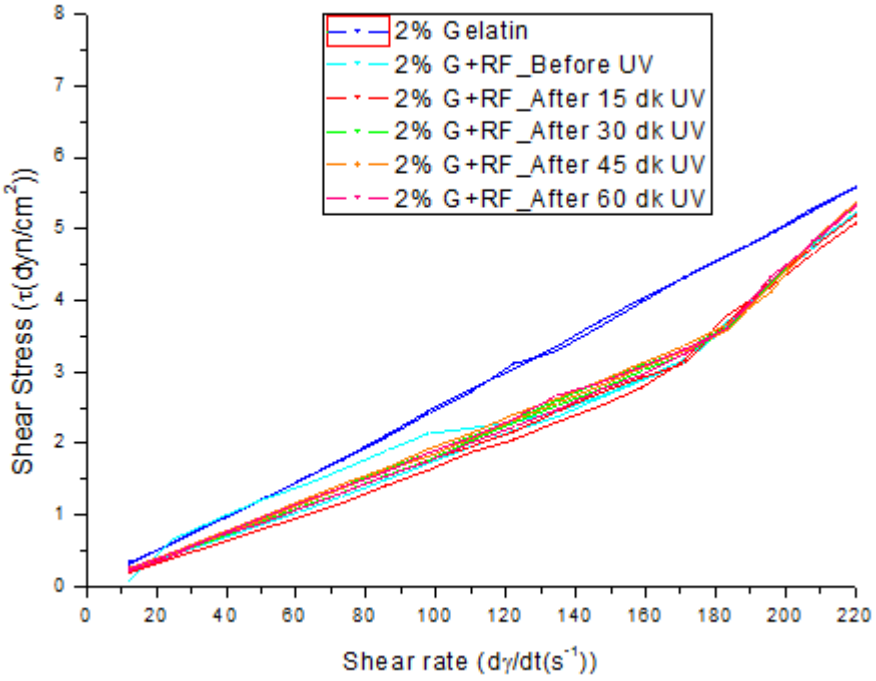



**Figure 7.4:** Shear stress versus shear rate graph for 2% gelatin based-riboflavin solutions before 370 nm UV and after various time.

According to Figure 7.4 addition of riboflavin and photoreaction of gelatin based-riboflavin solutions at various time intervals changed 2% pure gelatin behavior Newtonian to non-Newtonian behavior which was Dilatant or shear thickening. Altering time intervals which were applied 2% gelatin based-riboflavin solutions did not impact on the flow behavior which was shown in Figure 7.4 as different colors but one on the top of the other. Transforming Newtonian to Dilatant behavior can be explained that solution form of pure gelatin changed colloid form as a result of chemical reaction between riboflavin and gelatin. Moreover, riboflavin changed flow distribution of gelatin as a result of increasing rigidity of solution. However, the influence of photoreaction was not observed that is no difference on flow curves and internal mechanical features of solution before and after various time UV radiations.

Impact of glucose on gelatin solution was investigated for several concentrations of glucose such as 1%, 2%, 3%, 4% and 5% which was shown in Figure 7.5. First of all, 5% pure gelatin was selected as a based and various concentrations of glucose were added and altering of flow curves was obtained.

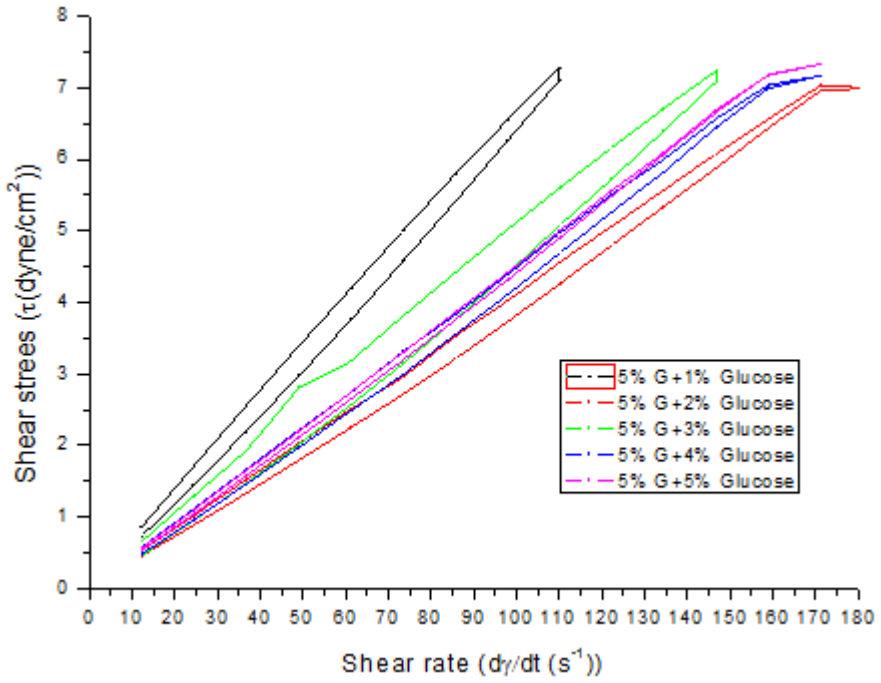



**Figure 7.5:** Shear stress versus shear rate graph for various concentration of 5% gelatin based-glucose solutions.

5% gelatin solutions were most intensive one and showed thixotropic behavior which was also non-Newtonian flow behavior. Addition of glucose reduced the hysteresis area as a result of composition of chains between glucose and gelatin and decreasing lack of joints between materials. Moreover, the internal energy emerged after chemical reaction was used to minimize time taken to move from any one state of microstructure to another and back again. The viscosity of gelatin based-glucose solution was decreased due to glucose acting as a plasticizing force between gelatin molecules. Moreover there was competition between gelatin and glucose molecules for water which was used to tenderize and swell molecules at high glucose concentration. However at low glucose concentration, there was not competition between molecules so that viscosity of solution increased.

To understand impact of glucose on gelatin solution, 2% gelatin solution was used as based solution secondly. Similar with 5% most intensive gelatin solution, various glucose concentrations were added to pure 2% gelatin solution one by one and viscosity of each solution were measured as shown in Figure 7.6.

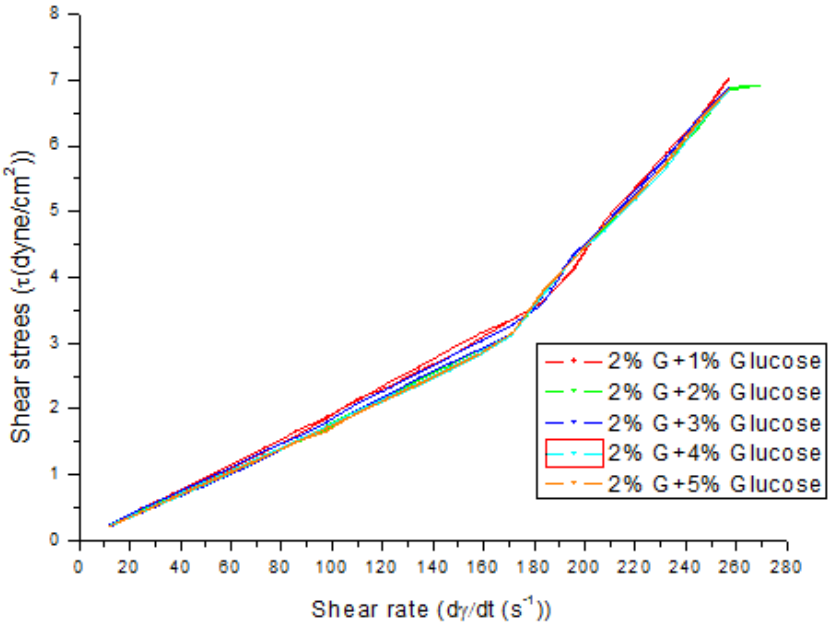



**Figure 7.6:** Shear stress versus shear rate graph for various concentration of 2% gelatin based-glucose solutions.

2% pure gelatin solution demonstrated Newtonian behavior as shown in Figure 7.2. However several concentrations of glucose were added to gelatin, behaviors of solutions altered and turned to non-Newtonian Dilatant behavior. As mentioned in 5% gelatin based-glucose solutions, glucose act as plasticizing force between gelatin molecules. On the other hand, as a result of glucose-gelatin reaction, hydrogen bonding between molecules increased and gelatin solution unfolded and solution turned to plastic.

Besides the effects of glucose, effects of NaCl at various concentrations were investigated during this study. 2% and 5% gelatins were selected as based solution and 1%, 2%, 3%, 4% and 5% concentrated NaCl were added one by one. First of all effect of NaCl on most intensive gelatin solution which was 2% was analyzed shown in Figure 7.7.

Pure gelatin solutions were shaped by physical cross-linking of the polymer chains. These polymer chains shaped an interconnected three dimensional network which was constructed into triple helices through inter-molecular hydrogen bonding. Moreover, polymer chains have positively and negatively charged sites along its length. The electrostatic interaction between these charged sites was important on the solution stability.

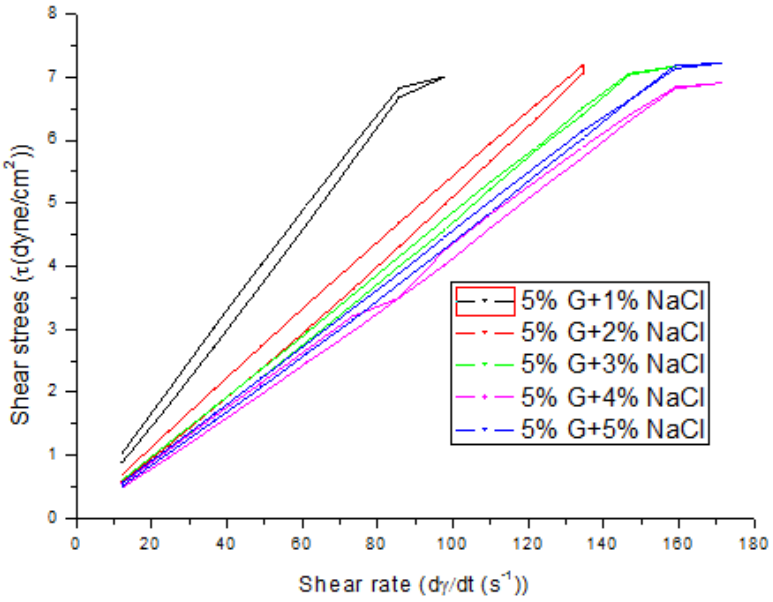



**Figure 7.7:** Shear stress versus shear rate graph for various concentration of 5% gelatin based-NaCl solutions.

The difference of electrostatic interaction between charged sites of gelatin structure caused different solution strength and other mechanical properties like viscosity of solution shown in Figure 7.7. The strength of gelatin solution decreased when a strong electrolyte such as NaCl were added to 5% pure gelatin solution. Also solution strength decreased as a result of increasing NaCl concentration. Inflection on shear rate of solutions could be explained by $Na^+$ and $Cl^-$ ions cover the charged sites of gelatin solution therefore electrostatic interaction decreased as a result of increasing concentration. However still there was not exact theoretical explanation of these inflections [20].

As mentioned previous, 2% pure gelatin solution demonstrated Newtonian behavior, at the end part, the effects various concentrations NaCl on 2% pure gelatin solutions were analyzed which was shown in Figure 7.8.

As mentioned, 5% gelatin based-NaCl solutions, gelatin-NaCl molecules interaction was electrostatic interaction which affected the solution strength beside concentration of NaCl growth. The 2% gelatin based-NaCl solutions demonstrated non-Newtonian Dilatant behavior when 2% pure gelatin demonstrated Newtonian. Electrostatic interaction between gelatin-NaCl molecules caused plasticize effect on solution but

increasing the concentration of NaCl did not significant impact on solution behavior due to electrostatic interaction was not strong as chemical forces.

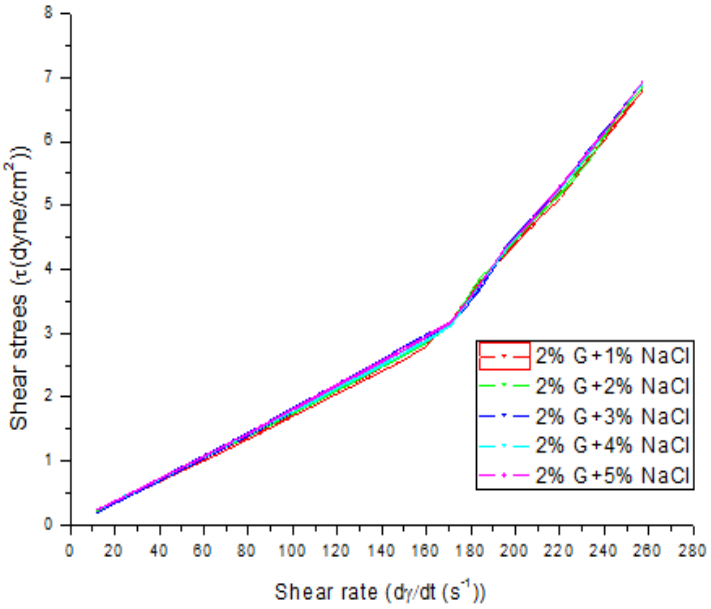



**Figure 7.8:** Shear stress versus shear rate graph for various concentration of 2% gelatin based-NaCl solutions.

Consequently, in addition to the shear stress-shear rate graphs drawing and analyzing appropriate mathematical models for each solution flow was determined and shown in Table 7.1

**Table 7.1:** Mathematical models for all solutions.

| Solution | Model | | |
|---|---|---|---|
| | Material Type | Mathematical model | Equation |
| 5% pure gelatin | Thixotropic | Bingham | $\tau = 0.35266 + 0.10915\dot{\gamma}$ |
| 3.3% pure gelatin | Thixotropic | Bingham | $\tau = 3.74191 + 0.01216\dot{\gamma}$ |
| 2% pure gelatin | Newtonian | Newtonian | $\tau = 0.02545\dot{\gamma}$ |
| 1.42% pure gelatin | Dilatant | Power Law | $\tau = 0.01015\,\dot{\gamma}^{\,1.5172}$ |
| 1% pure gelatin | Dilatant | Power Law | $\tau = 1.6582\dot{\gamma}^{\,1.1633}$ |
| 0.6% pure gelatin | Dilatant | Power Law | $\tau = 0.2074\dot{\gamma}^{\,1.00452}$ |
| 5%G+RF before UV | Bingham plastic | Bingham | $\tau = 0.23793 + 0.05143\dot{\gamma}$ |
| 5%G+RF after UV | Bingham plastic | Bingham | $\tau = 0.17248 + 0.534\dot{\gamma}$ |
| 5%G+1% Glucose | Thixotropic | Bingham | $\tau = 0.02215 + 0.06558\dot{\gamma}$ |
| 5%G+2% Glucose | Thixotropic | Bingham | $\tau = 0.06758 + 0.04069\dot{\gamma}$ |
| 5%G+3% Glucose | Thixotropic | Bingham | $\tau = 0.0834 + 0.0492\dot{\gamma}$ |
| 5%G+4% Glucose | Bingham plastic | Bingham | $\tau = 0.04634 + 0.004403\dot{\gamma}$ |
| 5%G+5% Glucose | Bingham plastic | Bingham | $\tau = 0.0295 + 0.04503\dot{\gamma}$ |

**Table 7.1 (continue):** Mathematical models for all solutions.

| Solution | Model | | |
|---|---|---|---|
| | Material Type | Mathematical model | Equation |
| 5%G+1% NaCl | Thixotropic | Bingham | $\tau = 0.100004 + 0.07592\dot{\gamma}$ |
| 5%G+2% NaCl | Thixotropic | Bingham | $\tau = 0.0410 + 0.05312\dot{\gamma}$ |
| 5%G+3% NaCl | Bingham plastic | Bingham | $\tau = 0.0135 + 0.04761\dot{\gamma}$ |
| 5%G+4% NaCl | Bingham plastic | Bingham | $\tau = 0.04883 + 0.04293\dot{\gamma}$ |
| 5%G+5% NaCl | Bingham plastic | Bingham | $\tau = 0.021 + 0.0448\dot{\gamma}$ |
| 2%G+RF before UV | Dilatant | Power Law | $\tau = 1.65599\,\dot{\gamma}^{\,1.30914}$ |
| 2%G+RF after UV | Dilatant | Power Law | $\tau = 1.70162\,\dot{\gamma}^{\,1.43131}$ |
| 2%G+RF after 15 min. UV | Dilatant | Power Law | $\tau = 1.70313\,\dot{\gamma}^{\,1.50669}$ |
| 2%G+RF after 30 min. UV | Dilatant | Power Law | $\tau = 1.99451\,\dot{\gamma}^{\,1.7579}$ |
| 2%G+RF after 45 min. UV | Dilatant | Power Law | $\tau = 1.86625\,\dot{\gamma}^{\,1.58604}$ |
| 2%G+RF after 60 min. UV | Dilatant | Power Law | $\tau = 1.70162\dot{\gamma}^{\,1.43131}$ |
| 2%G+1% Glucose | Dilatant | Power Law | $\tau = 1.78265\dot{\gamma}^{\,1.49809}$ |
| 2%G+2% Glucose | Dilatant | Power Law | $\tau = 1.88741\dot{\gamma}^{\,1.70146}$ |
| 2%G+3% Glucose | Dilatant | Power Law | $\tau = 1.66478\dot{\gamma}^{\,1.41999}$ |
| 2%G+4% Glucose | Dilatant | Power Law | $\tau = 1.50433\dot{\gamma}^{\,1.25482}$ |
| 2%G+5% Glucose | Dilatant | Power Law | $\tau = 1.49135\dot{\gamma}^{\,1.25155}$ |
| 2%G+1% NaCl | Dilatant | Power Law | $\tau = 1.49282\dot{\gamma}^{\,1.26206}$ |
| 2%G+2% NaCl | Dilatant | Power Law | $\tau = 1.48658\dot{\gamma}^{\,1.24191}$ |
| 2%G+3% NaCl | Dilatant | Power Law | $\tau = 1.57593\dot{\gamma}^{\,1.32982}$ |
| 2%G+4% NaCl | Dilatant | Power Law | $\tau = 1.4391\dot{\gamma}^{\,1.6695}$ |
| 2%G+5% NaCl | Dilatant | Power Law | $\tau = 1.57652\dot{\gamma}^{\,1.31612}$ |

### 7.2 Tensile Test of Films

Mechanical testing was performed to evaluate fundamental properties of materials as well as to control the quality of materials. Materials which were subjected to a load have to be strong and rigid enough to withstand the external forces. One of the basic tests to evaluate rigidity and strength of materials is tensile test which was performed during experimental study. The basic mechanical parameters of materials such as films which were designed in this thesis experimental part were determined by tensile test. Stress-strain curve was obtained for each film using results of tensile test. For each film, the dimensions of test sample were 26-28 mm length, 8-10 mm width and 0.06-0.2 mm thickness. 500N load cell was used with the tensile test machine with the cross head speed of 0.05mm/s for the each test sample. In addition to obtain stress-strain curve, modulus of elasticity and elastic potential energy per unit volume were evaluated by stress-strain diagram of each film.

First of all pure gelatin films were tested at tensile test machine at the room temperature and their stress- strain relations are shown in Figure 7.9 for unit cross-sectional area instead of load and strain graph.

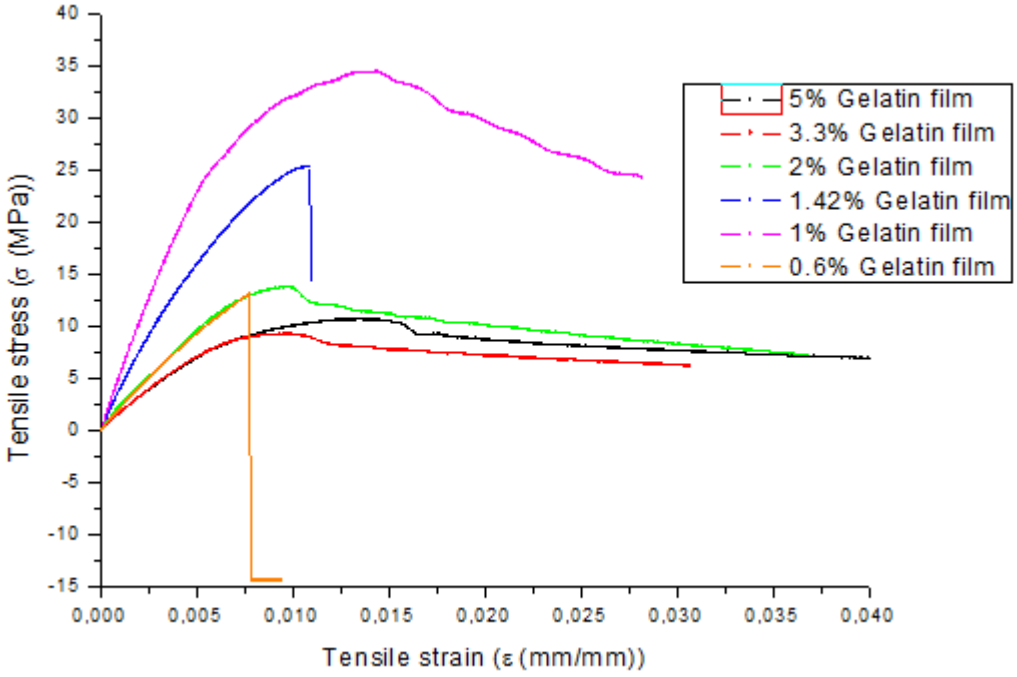



**Figure 7.9:** Tensile stress versus tensile strain for various concentrations of pure gelatin films.

According to Figure 7.9, tensile strength of pure gelatin films decreased with respect to increase of gelatin concentrations. Most intensive concentrations of gelatin films such as 5%, 3.3% and 2% were ductile and least intensive concentrations were brittle. This is may be caused by the property of gelatin molecules to recover the triple helix structure of collagen, having a more organized network at intensive form. Elongation is another important parameter in the application of polymer. Observing the figure, it was seen that the value of elongation decreasing with the increasing value of concentration and so happened due to cross linking.

Beside the effect of concentration on mechanical properties, the effects of ingredients such as RF, glucose and NaCl and effects of photoradiation this was researched using UV radiation were investigated. As mentioned viscosity measurement of solution, 5% and 2% gelatins were selected as a based material. Then RF, various concentrations of glucose and NaCl were added to these based gelatins one by one

that was explained in preparation of films part. Moreover, all the tensile test measurements were done in the same conditions such that $24^0$ C room temperature.

First of all the effect of UV sensitive riboflavin was tested by using 5% concentration of gelatin. The Figure 7.10 indicates the tensile test result of gelatin based RF films.

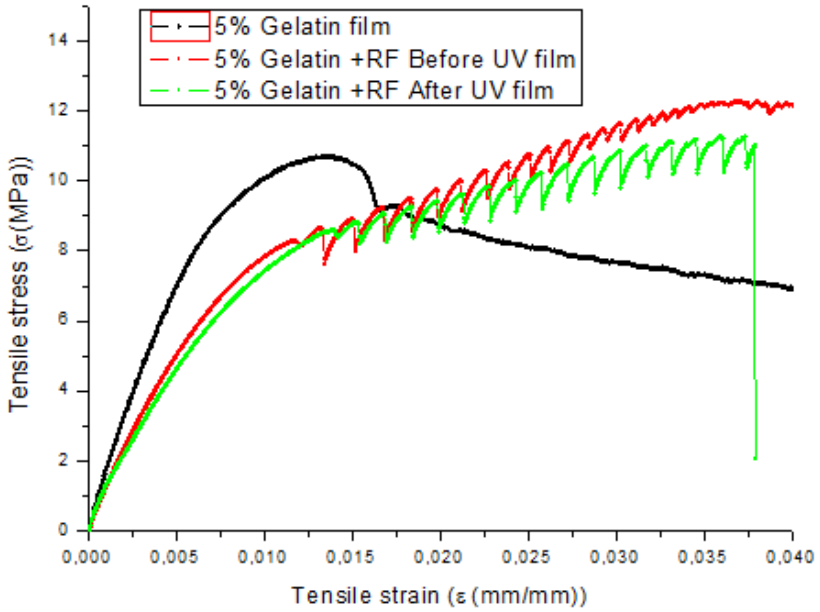



**Figure 7.10:** Tensile stress versus tensile strain for 5% gelatin and After-Before UV RF films.

According to Figure 7.10 elasticity of films increased when riboflavin was added and there was no difference between UV absorption of film and before UV film both of them ductile materials but had lower elastic limit than pure 5% gelatin film. As a result of moving on gear at test machine there were helicline on UV film curves. Tensile strength of pure gelatin film was higher due to degradation of the polymer chains and the films became soft and ductile.

Beside the most intensive concentrated gelatin film, the effect of UV absorption was investigated by using 2% gelatin and UV sensitive riboflavin films. Results of UV absorption effect on elasticity of films are shown in Figure 7.11.

Elastic characteristics of pure gelatin film and gelatin based-RF films were seen similar but the tensile strength and elongation values were seen different considerably. In case of pure gelatin film tensile strength value was 14 MPa but RF was added to gelatin during casting varying and found to decrease the tensile strength

significantly which may be RF was acting as filler between inter molecular and inter chain spaces hindering the helix structure of gelatin.

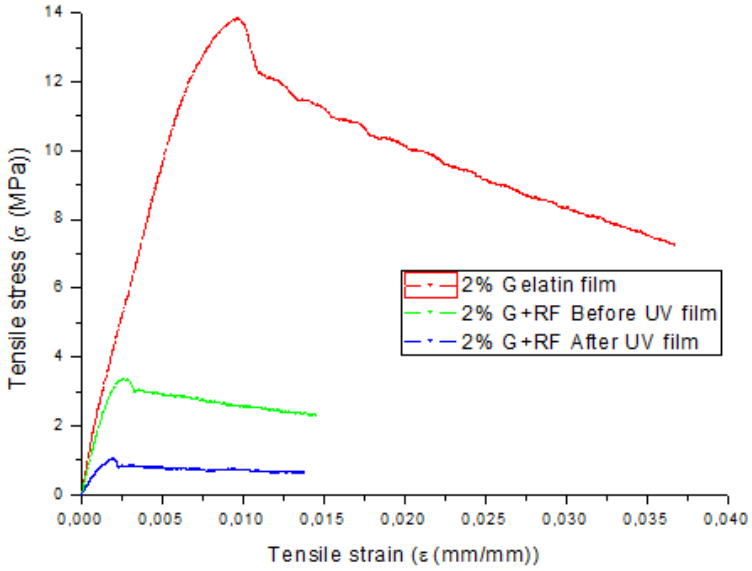



**Figure 7.11:** Tensile stress versus tensile strain for 2% gelatin and after, before UV absorption gelatin based RF films.

Besides the riboflavin effect investigation, the effects of glucose were investigated by using 5% pure gelatin and various concentration of glucose. This investigation was characterized at Figure 7.12.

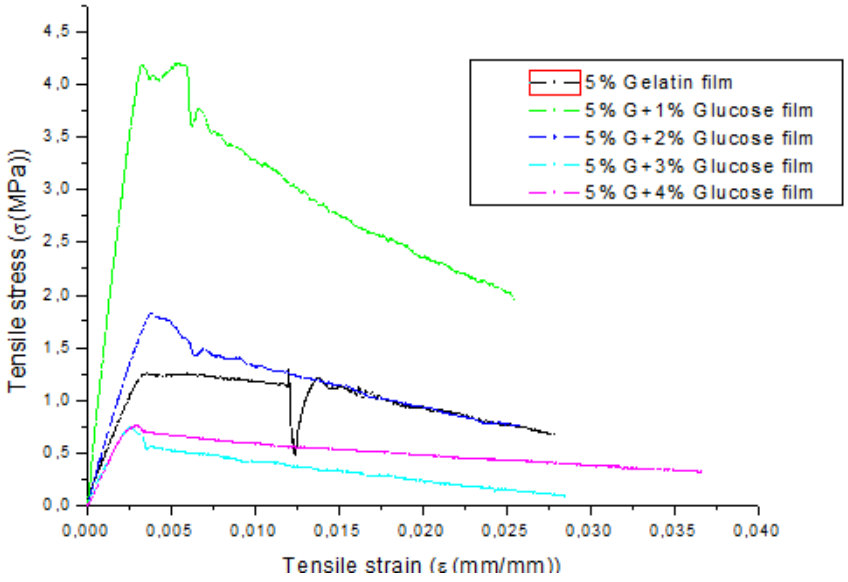



**Figure 7.12:** Tensile stress versus tensile strain for 5% gelatin and various concentration of gelatin based glucose films.

In case of gelatin based-glucose films, tensile strength value reached a maximum at 1% glucose concentration then tensile strength decreased further with increasing concentration of glucose. This could be explained by higher concentration might have caused chain scission due to the breaking of the polymer chains. Moreover, glucose has plasticizing effect on gelatin structure which made the films comparatively ductile.

In addition to glucose, the effect of NaCl also investigated in this thesis so that different concentrations of NaCl and 5% gelatin films were tested and results shown in Figure 7.13.

Although all NaCl concentration demonstrated similar mechanical characteristic, tensile strength reached maximum value at 1% NaCl concentration and then tensile strength decreased with increasing concentration as well as gelatin based-glucose films.

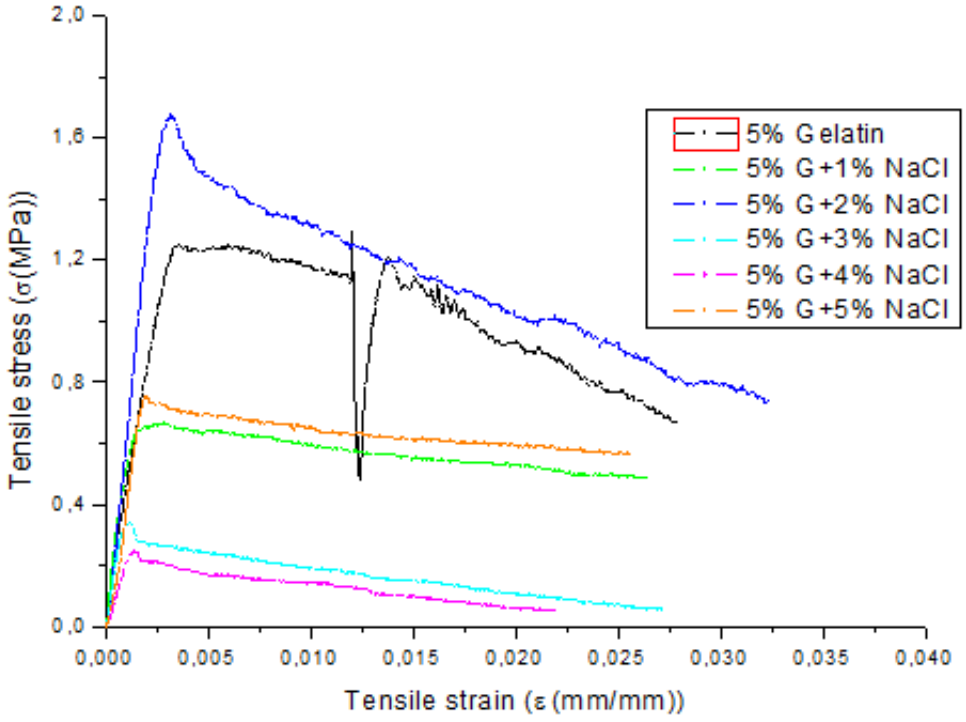



**Figure 7.13:** Tensile stress versus tensile strain for 5% gelatin and gelatin based NaCl films.

The strength of gelatin based-NaCl films decreased due to lack of interaction between gelatin molecules.

At the end the Young modulus and Elastic Potential Energy per unit volume for all films were determined by using the slope of tensile stress versus tensile strain graph and integrated it which is shown in Table 7.2.

As compared to the Modulus of Elasticity values of each film, the samples in this project seemed to have regarded not linear values with respect to concentration of gelatin. This difference might be as a result of the difference in the dryness of the films. The result from these tests were not conclusive in order to get a better idea of how concentration and other ingredients affects the mechanical properties of gelatin new experiments that take into consideration of concentration of gelatin and film dryness must be carried out.

**Table 7.2:** Elasticity modulus and Elastic potential Energy per unit volume values of all films.

| Film | Elasticity Modulus (MPa) | Elastic Potential Energy per unit volume $(\frac{MJ}{m^3})$ |
|---|---|---|
| 5% pure gelatin | 1447.5236 | 0.0214 |
| 3.3% pure gelatin | 1275.1455 | 0.0208 |
| 2% pure gelatin | 1768.1729 | 0.0406 |
| 1.42% pure gelatin | 3376.7762 | 0.0804 |
| 1% pure gelatin | 4495.4408 | 0.0838 |
| 0.6% pure gelatin | 1710.788 | 0.0503 |
| 5% G+RF Before UV | 968.4946 | 0.0296 |
| 5% G+RF After UV | 816.7392 | 0.0045 |
| 5% G+1% Glucose | 1360.1385 | 0.0058 |
| 5% G+2% Glucose | 507.9917 | 0.0029 |
| 5% G+3% Glucose | 309.8158 | 0.00074 |
| 5% G+4% Glucose | 316.8561 | 0.00072 |
| 5% G+1% NaCl | 618.4832 | 0.0023 |
| 5% G+2% NaCl | 387.1744 | 0.00024 |
| 5% G+3% NaCl | 205.85 | 0.00018 |
| 5% G+4% NaCl | 435.866 | 0.00051 |
| 5% G+5% NaCl | 471.4591 | 0.00043 |
| 2% G+RF Before UV | 1769.1214 | 0.0018 |
| 2% G+RF After UV | 750.4179 | 0.00031 |

## 7.3 Conclusion

The viscosity values of all solutions in this master thesis were investigated to being Newtonian or Non-Newtonian fluid and their mathematical models to determine flow behavior were shown. These solutions generally indicated that Newtonian, Dilatant and Plasticity characteristic. Several concentrations of pure gelatin solutions such as 5%, 3.3%, 2%, 1.42%, 1% and 0.6% were measured and analyzed. After analyzing

of shear stress versus shear rate graph, two concentrations of pure gelatin solutions which 5% was most concentrated one and 2% which showed Newtonian behavior, were chosen to observe influence of RF, glucose and NaCl. According to measurement of viscosity at constant speed, all solutions viscosity values were determined.

The viscosity measurement and graphical analyses of pure 5% gelatin solution demonstrated thixotropic characteristic. When RF was added to 5% gelatin solution, behavior of each fluid after-before UV 370 nm was observed. The results were showed that adding RF and UV absorption changed flow as thixotropic to plastic. Besides the RF affect, the influence of several glucose concentration and NaCl concentration were also measured. The flow types altered adding intensive concentrations of glucose and NaCl although diluted glucose concentration such as 1% and 2% demonstrated similar characteristic. Moreover yield point decreased this means that external forces decreased when RF, glucose and NaCl were added respectively and solution started to flow faster.

According to 2% concentration of pure gelatin`s experimental results and graphical analyses of viscosity measurement, pure 2% gelatin concentration was Newtonian material and obey the Newtonian behavior. However RF was added to pure 2% gelatin solution and UV absorbed nearly 2 hours solution become Dilatant. UV absorption time were changed to 15 minutes to 60 minutes increment of 15 minutes to observe affect of UV absorption time on 2% gelatin based RF solution indicated that solution behavior became Dilatant. In other words absorption time did not have significant affect on the behavior of solution. Affects of glucose and NaCl were also measured for various concentrations of glucose and NaCl solutions. 2% gelatin based glucose and NaCl solutions were Dilatant. All the result shows that external material such as RF, glucose and NaCl change solution types similarly.

According to viscosity measurement results which were gelatinous solution more suitable for different biological applications in terms of viscosity can be determined. In the literature review indicated that the viscosity of gelatin altered with concentration, pH and temperature for instance viscosity of 3.3% gelatin concentration at $40^0$C varied 44-55cP which was used in forming of two pieces hard capsules. In this thesis viscosity of pure 5% gelatin concentration at $24^0$C was

determined closely 14 cP and viscosity of pure 1% gelatin concentration at $24^0$C is determined closely 1.9 cP.

The pH level measurements of solutions were the range of biocompatibility pH tolerance limits. In consideration of the pH measurement, in actual, the human eye has a pH value that has a range between 7.1 and 7.39 depending on the movement of the eye [21]. To make a comparison, 2% gelatin and riboflavin solution after 15 minutes UV absorption had a close value as 6.63 with real value. Riboflavin was used in cross linking treatment of cornea disorder called kerataconus so that the measurement of gelatin riboflavin solution after UV absorption can give a support to study in this subject.

The output data of the Instron instrument for tensile testing included data for the stress and strain recorded for each film during testing. The stress and strain data was used to produce a Stress-Strain graph for each sample. Elastic limit point was determined and slope of the curve defined the modulus of elasticity for each film. Besides the modulus of elasticity mechanical characteristics of films such as ductile or brittle were explained according to graphical analysis. The elastic potential energy per volume of each film was investigated.

First of all pure gelatin films which were prepared by solutions at rate of 5%, 3.3%, 2%, 1.42%, 1% and 0.6% were tested with tensile testing machine which sets at crosshead speed of 0.05 mm/s, and load cell of 500 N. According to pure gelatin film tests and tensile stress strain graph, 1% gelatin film demonstrated highest elastic limit and yield point and this film was ductile. 0.6% and 1.42% films were brittle and 2% film showed least fracture point. High concentration films such as 5%, 3.3% and 2% were ductile materials.

The affect of RF and UV absorption were also tested using most concentrated gelatin film5%. The results of tests could be explained by 5% pure gelatin film showed higher elastic limit and yield point than 5% gelatin based RF films. Besides the RF, the impact of glucose were measured using the 5% gelatin based various concentrations of glucose films. The result indicated that 1% concentration of glucose had most elastic limit and it was brittle and 3% concentration of glucose had lowest elastic limit and it was ductile material. After that affect of NaCl was investigated using the 5% gelatin separately. Similar to 1% concentration of glucose

film, 1% concentration of NaCl had most elastic limit and it was ductile material. 3% and 2% concentrations of NaCl films had lowest elastic limit and 4%-5% concentrations of films had close elastic limit.

Also the affect of RF and UV absorption were tested on 2% gelatin films. 2% pure gelatin film had highest elastic limit and the elastic limit of before UV film was greater than after UV absorption. Before UV and after UV films had same fracture point at tensile strain values. This was explained by the dependence of mechanical properties of biopolymer films on the innate properties and how ingredients affect these properties. The mechanical structure of gelatin was altered easily according to external factors such as RF, glucose and NaCl; these were demonstrated one by one on graphical results. These results indicated that gelatin could be corporate with other biomaterials and possessed the desired mechanical properties need for biomedical applications.

All the important parameters to determine mechanical strength of films were investigated. Determination of mechanical properties of these biocompatible gelatinous films has an important role in developing biomaterials interact with biological systems as biomedical coatings. Moreover, to define physical properties of gelatin render it as candidate suitable for fabricating composite and tissue engineered scaffolds. This preliminary study suggested the diversity of gelatinous films that were appropriate to use various biomedical applications with respect to mechanical results.

## REFERENCES


**[1] James B. Rose, Settimio Pacelli, Alicia J. El Haj, Harminder S. Dua, Andrew Hopkinson, Lisa J. White and Felicity R.A.J. Rose.** (2014). *Gelatin-Based Materilas in Occular Tissue Engineering*, Materials, ISSN 1996-1944.

**[2] David F. Williams.** (2008). *On the mechanisms of biocompability,* Biomaterials, Volume 29, Issue 20, pp. 2.941-2.953.

**[3] Franz J. Ingelfinger.** (1980). *Dorland's Medical Dictionary*, 25th Edition, Saunders Press, ISBN-0030567440, 9780030567445-pp. 40.

**[4] D. Williams.** (2003). *Revisiting the definition of biocompability,* Medical Device Technology 14 pp. 8.

**[5] D. Mihov, B. Katerska.** (2010). *Some Biocompatible materials used in Medical practice,* Trakia Journal of Sciences, Vol8.

**[6] R. J. Young and P. A. Lovell.** (2011). *Introduction to Polymers*, 3th Edition CRC Press, ISBN-1439894159, 9781439894156.

**[7] M. J. Buehler, Y. Yung.** (2009). *Deformation and failure of protein materials in extreme conditions and disease*, Nature Materials, Vol8(3), pp-175-188.

**[8] R. Chandra, R. Rustgi.** (1998). *Biodegradable Polymers* Progress in Polymer Science Vol.23 pp.1273.

**[9] Masaru Tonako, Kazuhiro Sato, Erika Kitkaami, Shingo Kobayshi, Takoshi Hoshiba and Kazuki Fukushima.** (2015). *Design of biocompatible and biodegradable polymers based on intermediate water concept* Polymer Journal 47-114-121.

**[10] Rasuinder Singh Jessy, Mohammad Hakimi Ibrahim.** (2014). *Biodegradability and Biocompatibility of Polymers with Emphasis on Bone Scaffolding: A Brief Review,* International Journal of Scientific and Research Publications, Volume 4, Issue 2.

**[11] Changyun Pang, Gil-Yang Lee, Tae-il Kim, Sang Moon Kim, Hong Nam Kim, Sung-Hoon Ahn&Kahp-Yang Suh.** (2012). *A flexible and highly sensitive strain-gauge sensor using reversible interlocking of nanofibres*, Nature Materials 11, 795-801.

**[12] Jean-Peter Ylén.** (2001). *Measuring, modeling and controlling the pH value and the dynamic chemical state,* Helsinki University of Technology Control Engineering Laboratory Espoo, Report 127.

**[13] Covington A. K., Bates R.G., Durst R.A**. (1985). *Definitions of pH scales, standard reference values, measurement of pH and related terminology,* Pure & Applied Chemistry, Vol. 57, No. 3, pp. 531—542.

**[14] Tharwat F. Tadros.** (2003). *Rheology of Dispersions Principles and Applications,* ISBN: 978-3-527, pp. 38-47.

**[15] W. F. Hasford, P.Han Ed.** (1992). *Overview of Tensile Testing,* ASM International, pp. 1-24.

**[16] C. Mauli Agrawal, Joo L. Ong, Mark R. Appleford, Gapinath Mani.** (2014). *Introduction to Biomaterials-Basic Theory with Engineering Applications,* Cambridge University Press, pp. 20-26.

**[17] Devaralanda Annapurna Padmauathi.** (2011). *Potential Energy Curves & Material Properties,* Material Sciences&Application.

**[18] Naroyan R**. 2009 Edition *Biomedical Materials*, pp. 83-114.

**[19] Howard A. Barnes.** (1997). *Thixotropy-a review,* Journal of Non-Newtonian Fluid Mechanics,70 pp.1-33.

**[20]S. Chatterjee, H.B. Bahidar.** (2006). *Effect of salt and temperature on viscoelasticity of gelatin hydrogels*, J. Surface Sci. Tecnol., Vol 22, No.1-2 pp. 1-13.

**[21] Bonanno JA, Polse KA.** (1987). *Measurement of in vivo human corneal stromal pH.*

**Url-1**<*www.fy.chalmers.se/projects/biocompatiblematerials/publications/Biocomp*>, date retrieved 01.11.2015

**Url-2** <*scifun.chem.wisc.edu/chemweek/polymers/polymers.html/*>,

date retrieved 01.11.2015

**Url-3** <*en.wikipedia.org/wiki/polymer*>, date retrieved 01.11.2015

**Url-4** <*plc.cru.edu/tutorial/enhanced/files/polymers/synth/Synth.htm*>,

date retrieved 01.11.2015

**Url-5**
<*webeye.opth.uiowa.edu/eyeform/cases/60_Alphacor_SurgicalApproaches_Artificial-Cornea-Implant-htm*>,date retrieved 03.11.2015

**Url-6** <*www.visionexpowest.com/Press/Vision-Voice-Newletter/New-technology-for-Keratocunus*>, date retrieved 03.11.2015

**Url-7** <*www.discoveriesinmedicine.com/Apg-Bon/Artificial_Skin.html*>,

date retrieved 03.11.2015

**Url-8**<*www.burnresearchcenter.org/brcpublicwebsite/artificialskin.htm*>,

date retrieved 03.11.2015

**Url-9** <*www.viscopedia.com/basics/defining-viscosity*>, date retrieved 04.11.2015

**Url-10**<*www.donjins.com/sevice/file/Introduction%20to%20rheology.pdf*> ;

date retrieved 05.11.2015

**Url-11** <*www.viscopedia.com/methods/measuring-principles*>,

date retrieved04.11.2015

**Url-12**<*ocw.metu.edu.tr/file.php/43/Tension_Test_-_ekme-Deneyi.PDF*>,

date retrieved 06.11.2015

**Url-13** <*en.wikipedia.org/wiki/Elastic-energy#cite-ref-LL-1-1*>,

date retrieved 07.11.2015

**Url-14** <*www.gelatin.co.za/gltnl.html*>,date retrieved 07.11.2015

**Url-15** <*www1.lsbu.ac.uk/water/gelatin.html*>, date retrieved 08.11.2015

**Url-16**<*www.chemicalland21.com/lifescience/foco/RIBOFLAVIN.htm*>,

date retrieved 09.11.2015

**Url-17**<*internationalriboflavindatabase.weebly.com/chemical-propertiesformula-and-structure.html*>, date retrieved 10.11.2015

**Url-18**<*www.guidechem.com/products/42880-33-1.html*>, date retrieved 11.11.2015

**Url-19** <*hyperphysics.phy-astr.gsu.edu/hbase/organic/sugar.html*>,

date retrieved 11.11.2015

**Url-20** <*www.hms-beagle.com/7647-14-15*>, date retrieved 11.11.2015

**Url-21** <*en.wikipedia.org/wiki/Glucose*>, date retrieved 11.11.2015

**Url-22**

<*www.doe.mass.edu/mcas/student/2010/question.aspx?GradeID=1008&SubjectCode=bio_hsQuestionID=12090*>, date retrieved 11.11.2015

**Url-23** <*www.personal.kent.edu/~cearley/ChemWrld/jmol/crystals/crystals.html*>,

date retrieved 11.11.2015

**Url-24**<*study.com/academy/lesson/what-is-sodium-chloride-definition-structure-formula.htm*>, date retrieved 18.11.2015

**Url-25**<www.dummies.com/howto/content/ionic-bonds-why-and-how-ions-are-formed.html>, date retrieved 20.11.2015

**Url-26** <*en.wikipedia.org/wiki/Dilatant*>, date retrieved 20.11.2015

**CURRICULUM VITAE**

**Personal information**

First name / Surname: **HACİRE UĞUR**
Address : İnönü mah. Kartal cad. No:25 D:3 Kayışdağı-Ataşehir/İstanbul
Telephones : Gsm: 536-970-96-92
E-mail :hacireugur@yahoo.com
Nationality : T.C.
Date of birth : 10.10.1983
Date of place : İstanbul-Fatih
Gender : Female (Single)

**Work experience**

1–10/2005-4th Gathering in Istanbul for Freedom of Expression
-Position of interpreters

**Education and training**

1-Yeditepe University-Physics Department-2008
2-Arçelik Calibration Laboratory-2007(Practical Training)
3- Yildiz Technical University-Pedogogical Formation-2015

**Educational Achievements**

I.TUBİTAK SOLAR CAR RACE OF TURKEY-THIRDH

**Papers presented and published in the International Conferences**

1- H.Ugur,F.G. Acar "Investigation of Physical Deformation In Collagen Fibril Structure Using Finite Element Method","*9th Nanoscience and Nanotechnology Conference*" Pages:422-422(2013)
**2-** H.Ugur,F.G. Acar "Investigation of Viscosity Behaviour for Biocompatible Gelatinous","*11th Nanoscience and Nanotechnology Conference*" Poster Session 3/P22(2015)
**3-** H.Ugur, G.Akin Evingur,F.G. Acar "Investigation of Mechanical Properties for Biocompatible Gelatinous Films","*11th Nanoscience and Nanotechnology Conference*" Poster Session 2/P45 (201)

**Seminars and Educations I participated**

1)Nano7 Seminars Certificate
2)Fototrek Basic Photography Training-2008
3)Winter School on Quantum Field Theory(Feza Gursey Institute of Theoretical Physics-Istanbul/Turkey)-2014